

Cortical Bone Health in African Ancestry Men

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Abstract

As the population ages, more non-white men will develop osteoporosis and its associated fractures, yet little is known about skeletal aging in African ancestry men. Studies using quantitative computed tomography (QCT)-measured Bone Mineral Density (BMD) have exposed the incomplete nature of the current understanding of age-related bone loss. Furthermore, studies of the relationship between body fat and BMD have challenged the traditional paradigm that adiposity is beneficial for skeletal health. Moreover, even though cortical bone constitutes 80% of the skeleton, its epidemiology has rarely been described.

To address these knowledge gaps, this dissertation examined the rates of change and correlates for cortical thickness, periosteal and endosteal circumferences, and torsional bone strength (SSI) at the radius and tibia in middle-aged and older African ancestry men from the Tobago Bone Health Study over an average follow-up period of 6.2 years. Secondly, inflammation markers — high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL6) — were examined in relation to rates of change in cortical bone measures in both limbs. Lastly, total calf adipose tissue area (TAT) and its components, percentage subcutaneous (%SAT) and non-subcutaneous fat (%NSAT), were assessed in relation to changes in cortical bone measures at the tibia.

The main findings were: endosteal expansion outpaced periosteal bone formation, such that there was net cortical thinning and reductions in SSI in both limbs. HsCRP was not associated with rates of change in cortical bone measures, while higher baseline serum IL6 was significantly associated with slower rates of loss of cortical BMD and SSI at both limbs. Higher calf TAT was

associated with higher rates of cortical BMD loss at the tibia. Higher %SAT and % NSAT were associated with lower and higher rates of cortical BMD loss respectively Higher %SAT was also associated with significantly greater rates of periosteal expansion.

These findings have public health significance due to their focus on an under-studied population, detailed description of changes and correlates of cortical bone structure, particularly periosteal circumference, which is a promising therapeutic target for osteoporosis. Moreover, the relationship between adiposity and skeletal health is especially timely considering the alarming rates of obesity worldwide.

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Preface

Osteoporosis is generally considered a woman's disease and has largely been overlooked in men. In recent times, male osteoporosis has emerged as a public health condition of import; however, Caucasian men are over represented in osteoporosis studies. Fracture, the most serious preventable consequence of osteoporosis, occurs when bone strength has been compromised. Currently, bone density is assessed using dual X-ray absorptiometry (DXA), as a surrogate for bone strength. However, bone density captures only one facet of bone strength. More advanced imaging modalities like quantitative computed tomography (QCT) have allowed a three-dimensional visualization of the skeleton and already laid bare deficiencies in current theories and models of bone loss, even challenging the role of some predictors like higher body weight, which has long been considered protective of fractures. The objective of this dissertation was to use information from the Tobago Bone Health Study — a well-described cohort of Afro-Caribbean men — and describe the rates of change in QCT-derived measures of bone structure and bone strength. At the same time, the dissertation explored inflammation as a potential etiological mechanism, while also re-examining the relationship between higher body weight and bone health using QCT- measures of regional fat depots and bone structure information.

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I could not have completed my doctoral work had it not been for Maya and Hemant; Santhi and Hari; and Mamatha and Abhishek, who opened their homes to me.

Lastly, thank you to my family for always being there.

Nomenclature Used

DXA – Dual energy X-ray Absorptiometry

aBMD – Areal Bone mineral density

QCT – Quantitative Computed Tomography

BMD – Bone Mineral Density

SSI – Strength Strain Index

CrtThk – Cortical Thickness

PeriC – Periosteal Circumference

EndoC – Endosteal Circumference

IL6 – Interleukin 6

hsCRP – High sensitivity C-Reactive Protein

HSC – Hematopoietic Stem Cell

RANK – Receptor activator of nuclear factor kappa-B

RANKL – Receptor activator of nuclear factor kappa-B ligand

OPG – Osteoprotegerin

M-CSF – Macrophage Colony - Stimulating Factor

GH – Growth Hormone

IGF-1 – Insulin-like Growth Factor-1

FE – Finite Element

TA – Total Area

CA – Cortical Area

MA – Marrow Area

SM – Section Modulus

CSMI – Cross-sectional moment of inertia

pQCT – Peripheral Quantitative Computed Tomography

E2 – Estradiol

T – Testosterone

T2D – Type 2 Diabetes

ADT – Androgen Deprivation Therapy

HR – Hazard Ratio

SD – Standard Deviation

TNF α – Tumor Necrosis Factor- α

IL1 – Interleukin1

SHBG – Sex Hormone-Binding Globulin

OR – Odds Ratio

TbN – Trabecular number

TbSp – Trabecular Spacing

BMI – Body Mass Index

CVD – Cardiovascular Disease

vBMD – volumetric bone mineral density

TAT – total adipose tissue area

SAT – subcutaneous adipose tissue

VAT – visceral adipose tissue

TBF – Total Body Fat

FN – Femoral Neck

LS – Lumbar Spine

UDR – ultra distal radius

1.0 Introduction

Increased average life expectancy is among the greatest public health successes of the 20th century. All over the world, the average age of the population is rapidly increasing; by 2040, those aged 65 and over will constitute 14% of the population amounting to nearly 1.8 billion individuals. (1) Thus, the number of people affected by osteoporosis and consequently osteoporotic fractures is expected to rise; disproportionately affecting developing nations, where 50% of all hip fractures are expected to occur by 2050. (2) Approximately 70% of those affected by fractures are women and indeed osteoporosis has been extensively researched in women having long been considered a woman's disease. However, the number of men over the age of 50 suffering from osteoporotic fractures is considerable. (3) Approximately 40% of the estimated 9 million osteoporotic fractures in the year 2000 occurred in men. (4) Of these, about 45% occurred at sites usually affected by osteoporosis: 16% vertebral, 14% proximal femur, 10% distal radius, and 5% proximal humerus. (4) Furthermore, the mortality and morbidity due to hip fractures is higher in men. (5,6) Osteoporosis however continues to be underdiagnosed and undertreated in men due to a lack of clear and cost-effective clinical guidelines for men, even among those who clinically present with fractures. (7,8)

From a mechanical perspective, fractures occur when the load-bearing capacity of bones is exceeded. Even though Dual energy X-ray Absorptiometry measured (DXA-measured) low areal bone mineral density (aBMD) is the strongest risk factor for fracture, clinical studies evaluating pharmacological treatment strategies for osteoporosis have yielded results, where the reductions

in fracture risk did not correspond changes in aBMD. (9–11) These findings have drawn attention to biomechanical and morphological determinants of bone strength. Bone strength is determined by the size, shape and spatial orientation of bone material, (12) characteristics that DXA fails to capture. (13–15) Further, it cannot distinguish the trabecular and cortical compartments of bone that have distinct behaviors in relation to aging, diseases and treatments. (16–22) Quantitative Computed Tomography (QCT) overcomes the shortcomings of DXA and is also precise. (23) QCT studies have challenged traditional paradigms, which chiefly attribute age-related bone loss to a dramatic withdrawal of sex hormones in women around menopause and more gradual declines of the same in men. (24,25) By demonstrating loss of trabecular BMD beginning as early as the third and fourth decades in both men and women longitudinally and cross-sectionally, (26,27) Riggs *et al.* have highlighted the incomplete nature of existing models explaining bone loss. While biomechanical studies have shown that cortical bone determines the structural behavior of bone (28) and nearly 80% of all fractures are nonvertebral occurring at predominantly cortical sites, (29) cortical bone has rarely been assessed for its contribution to bone strength in epidemiological studies.

Most of the extant research on bone loss in men has largely focused on Caucasians. (30–36) Even though men of African ancestry have higher peak bone mass (PBM) (37,38) and a lower risk of osteoporosis compared to men of other ethnicities, rates of decline in BMD with aging may be comparable in Caucasian and African ancestry men. (39) The incidence of fractures in non-white subpopulations in the US is projected to increase nearly 2.7 times by 2025 (compared to 2005). (40) Moreover, in black Americans, the economic burden of fractures is expected to increase by almost 79%. (40) Despite these projections, information on skeletal health in African ancestry men is sparse.

By describing the patterns of cortical bone loss and its determinants in terms of bone structure rather than BMD alone, the objectives of this dissertation research were: to describe patterns of bone loss in an understudied population group — African ancestry men; to study inflammation as a potential etiological process underlying age-related bone loss — an extension of the association observed between biomarkers of inflammation and adverse cardiovascular and cognitive health outcomes; and to re-examine a heretofore protective factor against osteoporosis, namely higher body weight, in view of the alarming rates of global obesity.

Specific Aim 1: To describe the patterns of change and correlates of peripheral QCT (pQCT)-measured cortical torsional bone strength given by the polar strength strain index (SSI) and bone geometry given by cortical thickness (CrtThk), periosteal circumference (PeriC) and endosteal circumference (EndoC) at the proximal radius and tibia in African ancestry men aged 40 and over during a mean follow-up period of 6.2 years.

Specific Aim 2: To examine the association of baseline serum measures of biomarkers of inflammation — interleukin-6 (IL6) and high sensitivity C-reactive protein (hsCRP) — with pQCT-measured changes in cortical BMD, SSI, CrtThk, PeriC and EndoC at the proximal regions of the radius and tibia in African ancestry men aged 40 and over during a mean follow-up period of 6.2 years.

Specific Aim 3: To evaluate the relationship between pQCT-measured total and regional adipose tissue surface area at the calf (tibia) and pQCT-measured changes in cortical BMD, SSI, CrtThk, PeriC and EndoC in men of African ancestry aged 40 years and over with an average follow-up time of 6.2 years.

2.0 The Skeletal System

In addition to providing a framework for the body, the skeletal system also carries out vital functions like maintaining the delicate balance of calcium in the body, synthesizing blood cells, protecting organs, secreting hormones, and not least, enabling locomotion. Bones can be classified based on appearance, shape, and location. By gross appearance, bones can be classified as either cortical or trabecular. Cortical regions have densely-packed bone constituting 80% of the skeleton, whereas trabecular regions look mesh-like with interconnected cavities (Figure 1). By shape: bones can be long, short, flat, or irregular. By location: the axial skeleton comprises the skull, vertebral column, the sternum, and ribs; the remainder of the skeleton is referred to as the appendicular skeleton. The focus of this dissertation research has been cortical bone in the long bones of the appendicular skeleton, particularly the radius and tibia.

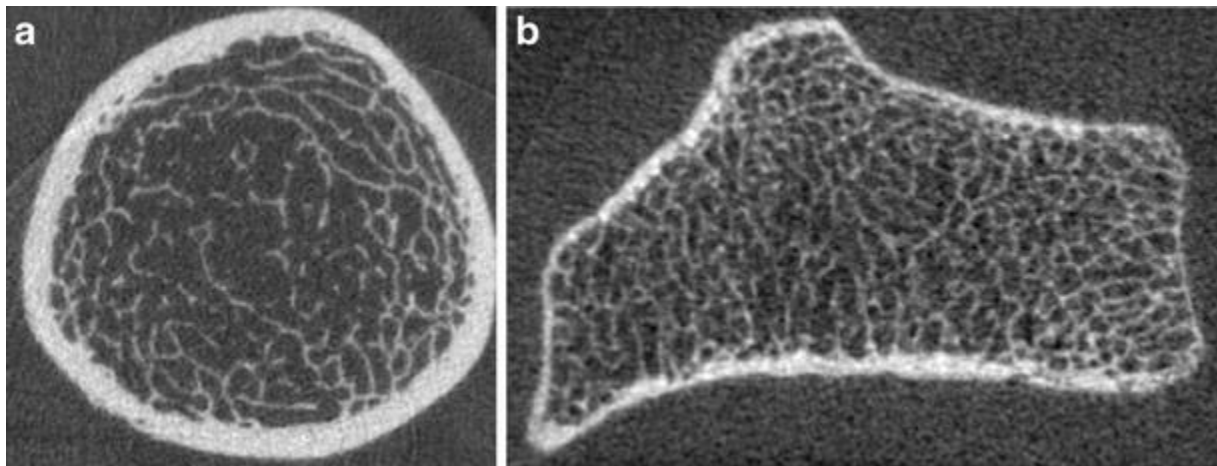


Figure 1 Cortical and trabecular compartments

High-resolution quantitative computed tomography scans showing one cross sections at the distal tibia (a) and radius (b). Reproduced with permission (41)

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2.1 Structure of Bones

Adult long bones have a cylindrical portion or diaphysis composed of cortical bone enclosing the marrow cavity and the epiphysis found at bone extremities, predominantly composed of trabecular bone. Longitudinal bone growth occurs at the growth plate or metaphysis found between the diaphysis and epiphysis in the growing skeleton. Externally, bones are covered by a membrane called the periosteum that has a fibrous outer layer and an inner cellular layer. Similarly, the endosteum, consisting of a cellular layer and lining cells, separates the intracortical and subcortical regions, and trabeculae from the marrow cavity. (41)

2.2 Composition and Organization of Bones

Bone is composed of the inorganic material hydroxyapatite and organic material predominantly consisting of collagen and three cell types: osteoblasts, osteocytes, and osteoclasts. Osteoblast is a bone-forming cell derived from the mesenchymal lineage that lays down bone matrix and aids in its mineralization. An osteocyte is derived from an osteoblast that has been entombed in mineralized bone. Osteoclast is a bone-degrading cell derived from the hematopoietic lineage, which dissolves mineralized bone and enzymatically degrades bone matrix. (42)

Osteoblasts produce bone matrix, which is 90% type I collagen. Bundles of type I collagen form interdigitating continuous sheets by means of hydrogen and covalent bonds. Hydroxyapatite mineralization occurs in gaps between collagen molecules, which in combination with the distensible collagen confers material stiffness. (43) Cortical bone is 60% mineral, 20% organic material and 20% water — a proportion crucial for balancing stiffness and flexibility. Osteoblasts

secrete bone matrix that hardens on calcification trapping osteoblasts in the process. The entrapped osteoblasts differentiate into osteocytes, which occupy spaces called lacunae and connect with other osteocytes by means of long cytoplasmic processes within spaces called canaliculi, forming a network akin to the neuronal network of the brain. In contrast to osteoblasts and osteoclasts, which are transient cells appearing only at skeletal sites undergoing remodeling, osteocytes outnumber osteoblasts by 10 to 1 and osteoclasts by a 1000 to 1. (44) Osteocytes are interconnected by the lacuno-canalicular network. Furthermore, they are also connected to endothelial cells in the bone marrow, and bone lining cells. (45) Thus, osteocytes are ideally placed to mediate bone homeostasis by regulating formation and resorption. (46)

Bone tissue is organized to have a lamellar structure. Lamellae are layers of bone matrix about 3 – 6 microns thick organized in a concentric fashion around a central canal. An osteon is the basic functional unit of cortical bone — a complex of concentric lamellae surrounding a central canal with blood vessels, nerve tissue, and endosteum. This organization greatly increases the strength of the bones. Both cortical and trabecular bone have a lamellar structure with differing arrangements (Figure 2). (42)

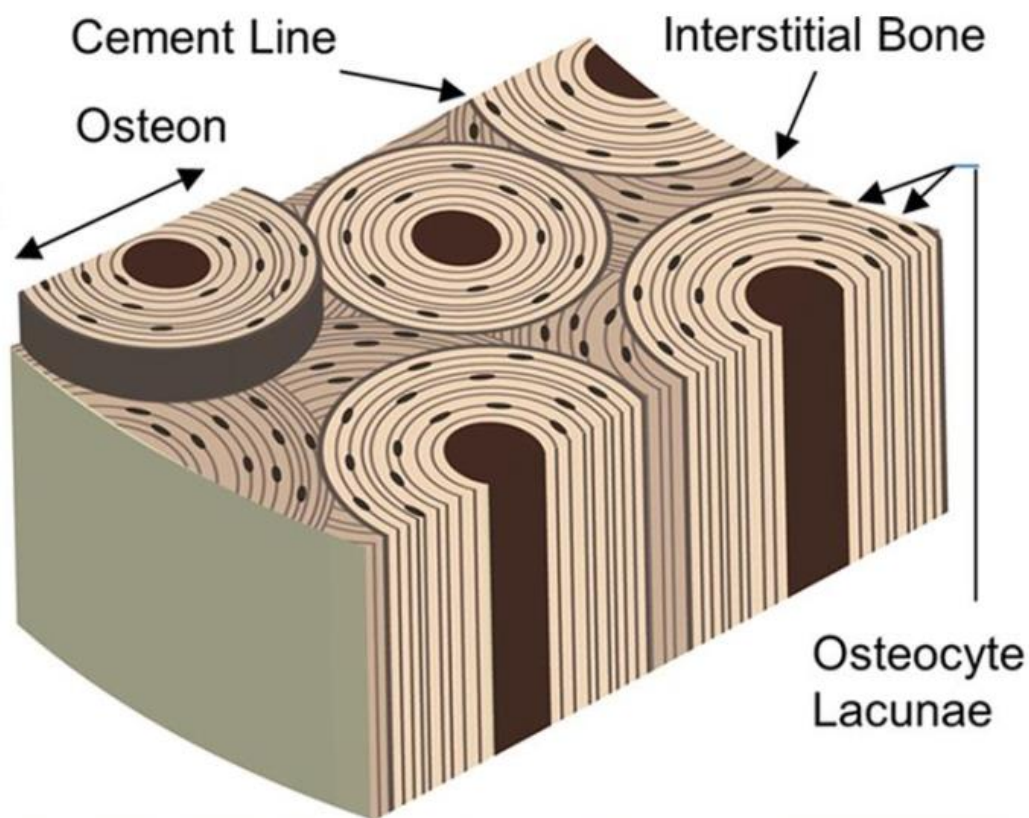


Figure 2 Osteon- the basic structural unit of bone

A complex of concentric lamellae surrounding a central canal with blood vessels, nerve tissue, and endosteum. Reproduced with permission from (47)

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2.3 Maintenance of the skeleton

The ability of the bones to perceive and respond to their loading environments by structural adaptations in the size and shape of their microarchitecture is referred to as the Mechanostat. (48) The Mechanostat response is governed by genetically-determined thresholds that can be modified by endocrinological factors. (49,50) Bone adaptation follows three rules: bone responds to dynamic rather than static loads; a short duration of loading is sufficient to trigger an adaptive

response; and bone cells get accustomed to routine loading signals leading to maintenance rather than increased bone mass. (51)

From fetal life till the completion of longitudinal growth by the end of the second decade, skeletal growth and maintenance occurs by the process of modeling. Modeling occurs throughout the skeleton resulting in alterations of skeletal size and macroarchitecture, often occurring at sites where resorption has not previously occurred. For the remainder of life, another distinct process called remodeling maintains the skeleton by repairing damaged bone, replacing old bone, facilitating vital functions like calcium homeostasis, and preserving the mechanical integrity of the skeleton. (52) Remodeling occurs in basic multicellular units (BMU) — an assembly of osteoclasts, endothelial cells, connective tissue, and osteoblasts — scattered across the skeleton, where resorption occurs over a period of three weeks followed by bone formation over a duration of three to four months. Remodeling is distinct in the cortical and trabecular compartments. Once a bone site has been identified for remodeling, lining cells separate and create a bone remodeling compartment (BRC) containing BMUs. Within this compartment, bone cells, endothelial cells and immune cells communicate with each other. Hematopoietic stem cells (HSC) gain access to the bone BRCs from nearby capillaries, differentiate into osteoclasts and bring about bone resorption. Subsequently, osteoblasts lay down new bone. (53) In bone areas undergoing resorption, osteoclasts lie in cavities on the bone surface, which upon coming in contact with the bone matrix, form a sealing zone with a ruffled border that binds tightly to the underlying bone matrix creating a specialized microenvironment for bone resorption to occur. The ruffled border is rich in material necessary for resorption. (42)

The key proteins regulating remodeling are: receptor activator of nuclear factor kappa-B (RANK), receptor activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegerin

(OPG). Their initial differentiation depends on macrophage colony-stimulating factor (M-CSF), which upregulates the expression of membrane-bound RANK on osteoclast precursors; RANK then combines with RANKL expressed by osteoblasts and osteocytes. RANK activation in concert with other factors increase the expression of genes leading to the final differentiation and fusion to form osteoclasts. RANK is tightly regulated by OPG, a decoy receptor resembling RANKL released by osteoblasts, which blocks osteoclastogenesis. Factors regulating bone remodeling have been summarized in figure 3.

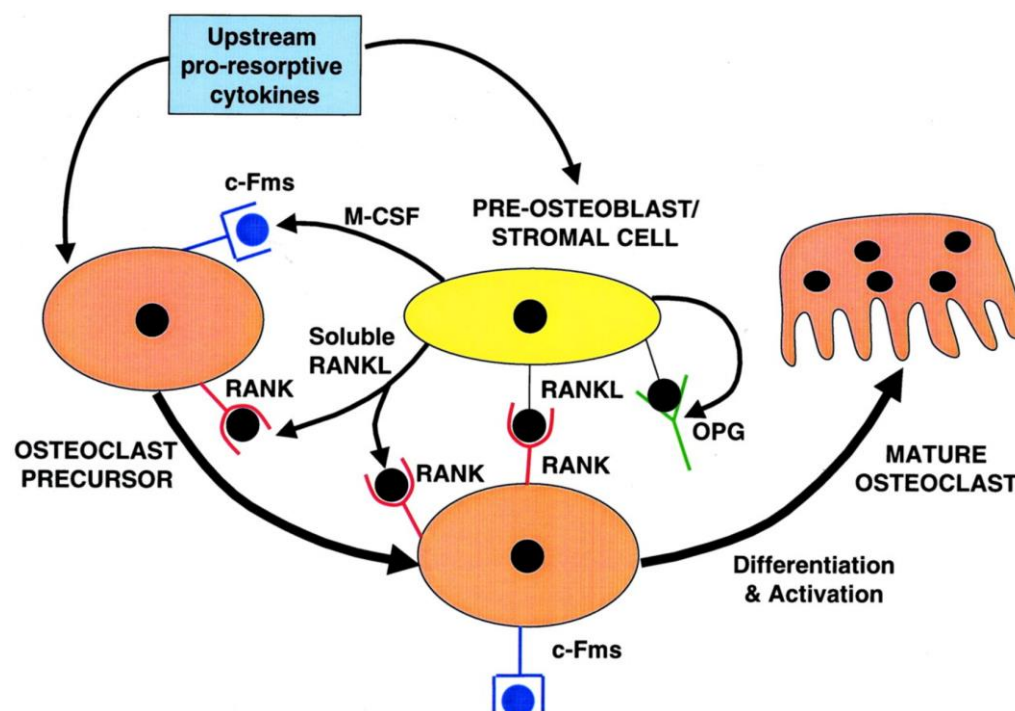


Figure 3 Regulation of Bone Remodeling

Reproduced with permission (54). RANK - receptor activator of nuclear factor kappa-B; RANKL - receptor activator of nuclear factor kappa-B ligand; OPG – Osteoprotegerin; M-CSF - Macrophage Colony Stimulating Factor; c-Fms - Colony-Stimulating Factor 1 receptor

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2.4 Origins of Bone Fragility

The prepubertal years are characterized by greater appendicular skeletal growth than axial skeletal growth in both sexes. (55,56) At puberty, the axial skeleton grows more rapidly; trabecular BMD increases by trabecular thickening. (57) During longitudinal growth, the bone balance in the BMU is positive as net bone gain occurs with each remodeling event. At the completion of growth, bone balance in the BMU is zero — the amount of bone resorbed and deposited is equal. (58) In the peri-pubertal period, cortical width increases by periosteal apposition in boys. (59–61) Androgens, growth hormone (GH), and insulin-like growth factor-1 (IGF-1) stimulate periosteal bone formation, while estrogens inhibit it. (61,62)

In adulthood, the purpose of remodeling is to maintain the skeleton by repairing damage incurred by repeated loading. (63,64) Microcracks damage the canalicular network leading to osteocyte apoptosis. (65) Osteocyte apoptosis occurs within three days of immobilization (66); it can also occur due to estrogen deficiency or corticosteroid therapy. (67,68) A dead osteocyte provides site-specific information for the initiation of osteoclastogenesis. (69)

Four age-related processes pertaining to bone remodeling can decrease bone strength. (69) Firstly, a reduction in periosteal bone formation at the tissue level. After the completion of longitudinal growth, periosteal bone formation decreases swiftly and continues at a modest rate through adulthood. (70–72) Secondly, there is a reduction in bone formation at the cellular level, within each BMU. (73,74) Thirdly, continued resorption occurs in the BMU. (68,75,76) Lastly, an increase in the rate of remodeling, particularly observed in women following menopause. (46) As a result of these processes the amount of bone formed is less than the amount of bone resorbed resulting in a negative net BMU bone balance. (69)

Periosteal bone formation determines the cross-section of the bone. Endosteal bone resorption determines cortical thickness. Bone loss is effectively the net result of periosteal bone formation and endosteal bone resorption during aging. Endosteal bone resorption is a function of the number of remodeling units and negative BMU bone balance. (77)

With its greater surface-to-volume ratio, age-related trabecular bone losses are higher as compared to cortical bone losses. (78) Men lose nearly 40% of their net trabecular bone before the age of 50, in comparison cortical losses are minimal before the age of 50. (79) However, through young adulthood, bone loss is less pronounced as the rate of remodeling is low. Decreased bone formation is the predominant abnormal remodeling process, and periosteal apposition offsets endosteal bone loss by favorably reconfiguring available bone mass to maintain biomechanical integrity. (69,72)

As the available trabecular surface decreases, cortical surface - to- volume ratio increases and intracortical and endocortical surfaces become available for remodeling. Consequently, cortical bone is trabecularized, cortical thinning occurs, cortical porosity increases. Thus, with increasing age, bone loss shifts more to the cortical compartment. (69) Cortical thinning, increased porosity and coalescing pores limit the bone's ability to prevent the propagation of microcracks that lead to fracture.

In summary, long bones increase in diameter as new bone tissue is added beneath the periosteum in a process called appositional growth. New bone formation at the periosteal surface is accompanied by bone removal occurring concurrently at the endosteal surface enlarging the marrow-filled region while keeping the bone light (Figure 4). (42)

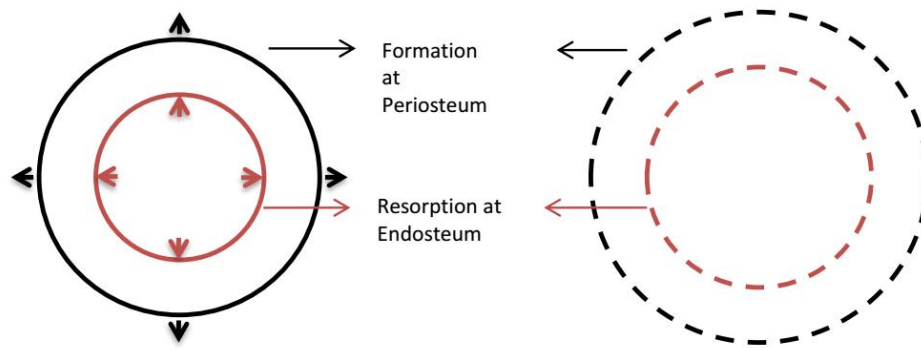


Figure 4 Periosteal bone growth and endosteal bone removal

Schematic representation of cross-section of a long bone. Endosteal expansion by bone resorption occurs concurrently with periosteal expansion by bone formation resulting in increased total bone area

2.5 Whole Bone Strength

Whole bone strength or the load-bearing capacity of the bone is determined by bone mass (size), the spatial distribution of bone material (shape and microarchitecture), and the intrinsic properties of the materials that comprise bone (density, matrix mineralization, collagen traits, and microdamage) (Figure 5). These three determinants of bone quality are in turn influenced by bone remodeling. Therefore, any disease or drug that affects bone remodeling ultimately affects bone strength. (80) Bone is living tissue, capable of self-repair and is constantly adapting to its environment. For example, in response to the increased mechanical loading on the dominant arms among tennis players, the bones increase in size. (81) Fractures occur when the load-bearing capacity of bones is exceeded. BMD, due its accessibility, is widely used as a measure of bone biomechanical competence and as a predictor of fracture risk. BMD-based risk assessment focuses on hip, spine, and the distal radius — all regions rich in trabecular bone. A large body of research

supports the association between osteoporotic fractures and BMD assessed at these sites. (13) However, there is considerable overlap in BMD among those who do and do not suffer fractures. (82) As noted earlier, BMD only represents one facet of whole bone strength.

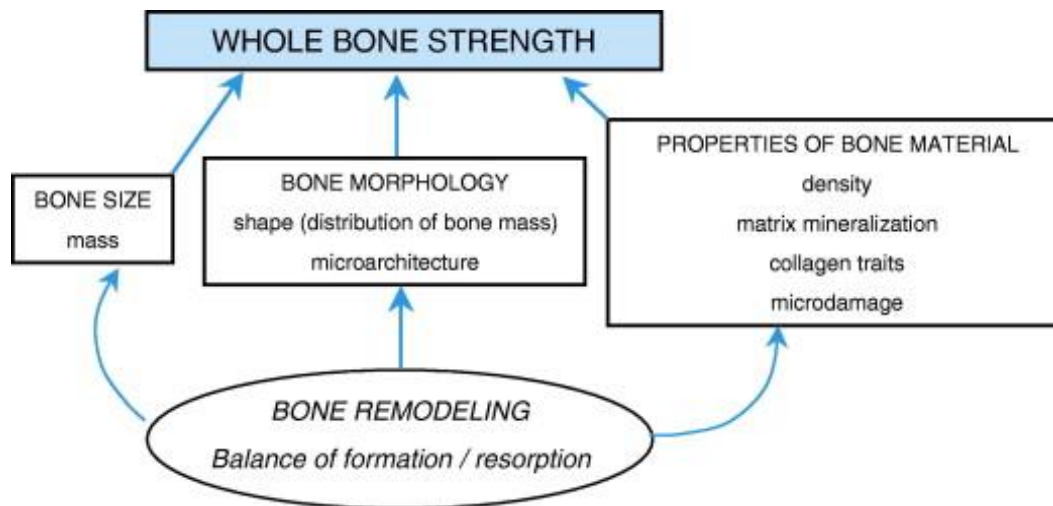


Figure 5 Determinants of bone strength
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2.5.1 Assessment of Bone Strength

Bone strength has mostly been measured using aBMD. The clinical gold standard for the assessment of BMD is still DXA. (83) DXA has several advantages: excellent in vivo precision, low radiation exposure, accuracy, short examination times and ability to predict fracture risk. (83–87) However, being a two-dimensional modality DXA cannot provide an estimate of volumetric BMD (vBMD) or distinguish between the trabecular and cortical bone compartments, which have distinct behaviors in relation to aging, diseases and treatments. (16–22) Thus, DXA is unable to capture redistribution of bone mineral in response to treatment; a limitation that precludes its use

in monitoring the response to bisphosphonates and selective estrogen receptor modulator drugs using aBMD. (9–11)

QCT overcomes the shortcomings of DXA and is also precise. (23) Indeed, QCT studies of age-related bone loss have challenged traditional paradigms by demonstrating loss of trabecular bone as early as in the 4th decade. (26,88) In addition to total, cortical and trabecular BMD, QCT also provides measures of bone strength and bone structure. Furthermore, information from these scans can be used to conduct simulations of bone loading under different conditions using finite element (FE) modeling. The following measures of structure and strength can be obtained from QCT depending on the site (also summarized in figure 6): cortical BMD (Cort BMD) in mg/mm^3 ; total area (TA) in mm^2 ; cortical area (CA) in mm^2 ; marrow area (MA) in mm^2 ; polar SSI in mm^3 ; CrtThk in mm; EndoC in mm; PeriC in mm; and section modulus (SM) in mm^3 . The QCT modality is not entirely free of errors: soft tissue thickness affects the measurement of trabecular BMD, cortical BMD and bone mineral content (BMC). However, these are not concerning under normal conditions.

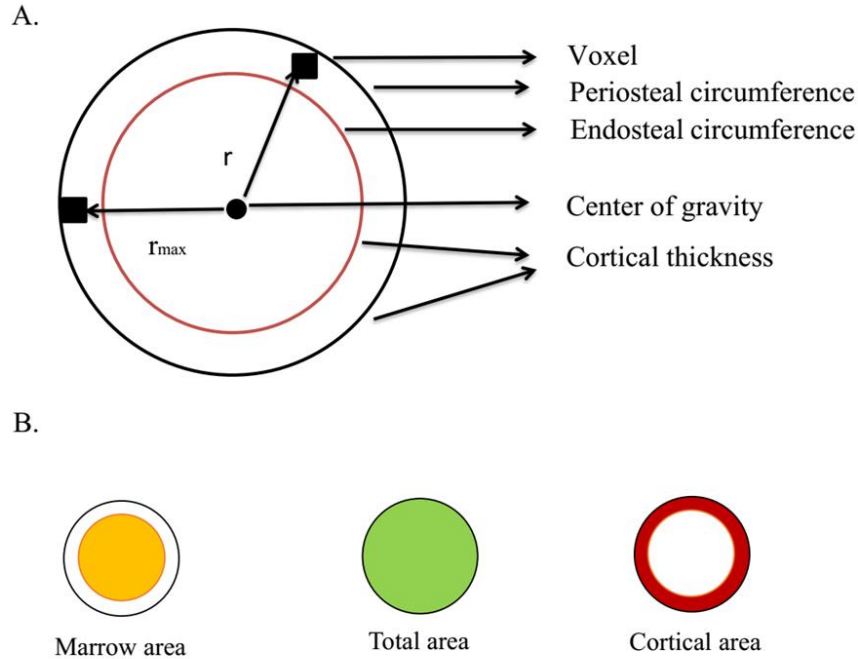


Figure 6 QCT-measures of cortical bone

A. Long bone cross-section showing QCT-measures of cortical bone.

CD_{vox} = cortical BMD in the voxel (mg/cm^3) ND = Maximum vBMD in humans under physiological conditions ($1200mg/cm^3$)

$$SSI = \sum (r^2 * a * CD_{vox}/ND) / r_{max}$$

B. Bone cross cross-section showing three bone surface area measures: Marrow area is another measure of endosteal expansion; Total area is another measure for periosteal expansion; and cortical area is another measure for cortical thickness.

2.6 Importance of Bone Structure

Bone's mechanical behavior is driven by its material and structural properties. The mineral component gives strength and stiffness to the bone. The collagen composition of bone influences its stiffness, but owing to its arrangement, mainly increases the toughness of the bone (the energy required to break it). The most efficient way for a bone to withstand the many stresses it is subject to is by distributing bone material away from its neutral axis, which leads to a marked increase in its resistance to bending and torsion. The property of distributing mass about the neutral axis of a structure is called cross-sectional moment of inertia (CSMI). (77)

Bone strength under compressive forces (F) is given by:

$$F = \sigma \cdot TA.$$

Under bending forces, the strength is given by:

$$M = \sigma \cdot (CSMI/r)$$

σ represents material strength. TA is the total cross-sectional area, CSMI represents the cross-sectional moment of inertia, r = distance from the center to the outermost point on the cross-section (Figure 8). Based on the above relations, strength depends on intrinsic material strength and TA or CSMI, which are in turn related to radius.

$$TA = \pi/4 (r_p^2 - r_e^2)$$

$$CSMI = \pi/4 (r_p^4 - r_e^4) \text{ where}$$

r_p is the periosteal radius

r_e is the endosteal radius

This relationship highlights the importance of the periosteal radius in bone strength. In summary, the CSMI is proportional to the fourth power of the radius of cross-sectional bone;

therefore, small increases in radius lead to large increases in resistance to bending (Figure 7A and 7B).

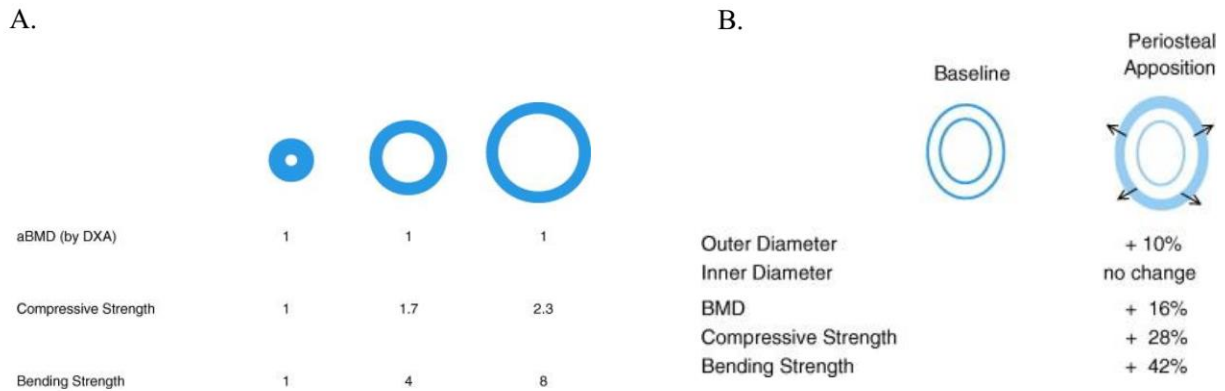


Figure 7 Importance of bone structure and CSMI

A. shows three individuals with the same BMD with differing bone distributions around the marrow cavity. B. illustrates the role of CSMI Reproduced with permission (12)

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Bones are dynamic structures constantly responding to their environment. During remodeling, mechanical loading on the skeleton increases periosteal bone in areas of high stress leading to large increases in mechanical strength. The aging appendicular skeleton is characterized by this redistribution of bone material at the periosteal surface to preserve bone strength in the face of age-related loss of BMD. (77) Studies have underscored the importance of bone geometry in preventing fractures: local cortical thinning and endosteal resorption was seen in femoral neck fractures (89); regions usually loaded during falling have thinned cortices at the femoral shaft. (90)

2.7 Importance of Cortical Bone

Osteoporosis research has focused mainly on bone loss at trabecular-rich regions, particularly vertebral bodies. (29,91,92) However, studying cortical bone is crucial considering: most of the skeleton consists of cortical bone; muscles are directly or indirectly attached to the periosteum, thus cortical bone predominantly carries out the load-bearing function of the skeleton (78); nearly 80% of all fractures are non-vertebral (29); occurring at predominantly cortical sites (29); most (about 70%) age-related appendicular bone loss is cortical (28,29); and, with advancing age, intracortical remodeling increases cortical porosity and compromises bone strength. (29,93,94)

2.7.1 Epidemiology of cortical bone structure and strength

Bone structure and strength have been assessed at cortical regions of the femur, radius, and tibia. Bone structure has been assessed in terms of periosteal expansion or the expansion of TA, endosteal expansion or expansion of MA, and cortical thinning or changes in CA or CrtThk. Similarly, bone strength has been assessed in terms of SM, CSMI or SSI, which represent changes in TA rather than CA. A summary of the studies examining these parameters is given in the appendices.

2.7.1.1 Cross-sectional studies of cortical bone structure and strength in men

Many early cross-sectional studies (90,95,96) were based on cadaveric bone specimens from predominantly white men. One such study of 38 US white males aged 20 – 99 years showed increases in CA, CSMI, and TA. Another study (95) of 93 Australian men aged 21 – 100 years

showed that: CA increased till the 7th decade, but declined subsequently; MA doubled over the age range studied; intracortical porosity increased from 4 – 6 % in young men to 9% in elderly men; Total sub-periosteal porosity only increased in men in their 80s from 25% in young men to 37%.

However, in recent times QCT has been employed in cross-sectional assessments of bone structure in larger samples. (97–100) Russo and colleagues (98) examined 512 men aged 20 – 102 years participating in the Invecchiare in Chianti (InCHIANTI) Study (101) from the Tuscany region in Italy. They reported that CA and minimum moment of inertia, at 38% tibia remained mostly unchanged across age groups.

Riggs and colleagues (97) studied 323 white men aged 20 – 97 years using data from the Rochester Epidemiology Project. (102) The participants underwent pQCT at the proximal and distal radius and tibia. They reported unadjusted and height-adjusted TA, MA, and CA values; bone strength indices were not reported. At the distal radius, TA and MA showed significant increases between age 20 and 90. Height-adjusted TA increased by 24% and height-adjusted MA expanded by 35%. On the other hand, at the proximal radius, height-adjusted TA increased by a lower magnitude of 15%. At the distal tibia, height-adjusted TA expansion and MA expansion was significant at 11% and 14% respectively. CA changed by 2% as an increase at the distal tibia and a decline at the proximal tibia; however, neither was significant.

In 230 Japanese men aged 30 – 84 years, Kaji and colleagues (103) studied age-related changes in radial structure at the 20% site. Age was significantly correlated with all measures of cortical structure and strength; the correlations were -0.0401, -0.414, 0.326, 0.25, and -0.368 for CA, CrtThk, EndoC, PeriC, and SSI respectively.

Sigurdsson and colleagues (99) examined 807 Icelandic men aged 67 – 93 as part of Age, Gene/Environment Susceptibility – Reykjavik Study (AGES – REYKJAVIK). Participants

underwent a single QCT of the lumbar spine, femoral neck and mid-femoral shaft. They reported midlife height and current weight adjusted TA, CrtThk, and strength indices – bending and compressive strengths. At the femoral shaft, TA increased by 2 – 3%/decade. Of the strength indices reported, bending strength at the femoral shaft significantly increased by 6.2%/decade.

Yuen and colleagues (100) examined a Chinese cohort of 620 men aged 20 – 98 using pQCT-measured bone structure and strength at the 33% radius and tibia. TA increased at the radius in young men and at the tibia in old men. Section modulus did not show any age-related changes except a decrease at the tibia in young men. Adjustment for weight did not alter the age-related trends in TA and SM across age-groups. Upon comparing men aged 60 and over to those under 60 years of age, CA and section modulus were significantly lower at the radius; however, no such differences were seen at the tibia. Compared to the radius, greater bone enlargement was seen and SM was better maintained at the tibia, particularly in older men. Overall, the tibia showed more trabecularization and bone enlargement.

Ward and colleagues (104) examined Caucasian men aged 40 – 79 years participating in the European Male Ageing Study (EMAS). They separately examined men from two study sites: Manchester and Leuven. A significant age-related decline in CrtThk and a significant increase in MA were reported at the mid-shaft of the radius in men from both study centers.

In summary, based on these cross-sectional studies, (95,96,98–100,103,104) periosteal expansion occurred at a rate of approximately 2%/decade, endosteal expansion at 7%/decade, and the resultant cortical thinning at 1%/decade at the shaft of the femur and tibia in men. (105) At the proximal radius, cortical thinning occurred at a rate of approximately 3%/decade. At the distal tibia, periosteal expansion and endosteal expansion occurred at a rate of 1%/decade. (97,100,105–

107) At the distal radius, age-related changes were similar to those at the distal tibia.
(97,100,104,105)

2.7.1.2 Longitudinal studies of cortical bone structure and strength in men

The sole longitudinal study of bone geometry using QCT in men to date was conducted by Lauretani and colleagues, (106) who examined 345 men aged 21 – 102 years participating in the InCHIANTI Study. As declining periosteal apposition with aging had been shown only in cross-sectional studies, (97,108–110) Lauretani and colleagues set out to demonstrate that osteoporosis was a result of the disequilibrium between periosteal apposition and endosteal resorption using a longitudinal design. (111)

Over a 6-year follow-up period: TA increased in men till the 7th decade; MA increased with aging; CA increased with age in young men, halted in the 6th decade, and declined subsequently. Overall, TA, indicative of periosteal apposition occurred at a rate of 1.2%/decade, CA, indicative of cortical thinning, increased at a rate of 0.6%/decade and MA, indicative of endosteal expansion increased by 3.7%/decade. (105,106) Their findings suggest a substantial loss of bone elastic stability, leading to decline in bone strength due to a reduction in cortical bone. (112)

2.7.2 Determinants of cortical bone structure and strength

Unlike the determinants of low aBMD, those of bone geometry and strength have not been well described. However, a few have assessed lifestyle and endocrine determinants of bone geometry. (103,113,114)

2.7.2.1 Lifestyle factors

In Japanese men aged 30 – 84, grip strength was significantly correlated with CA, CrtThk and SSI; the correlations were 0.225, 0.183 and 0.305, respectively. When comparing 21 men with vertebral fractures to 177 men without vertebral fractures, CA, CrtThk, and SSI were significantly greater among those without fracture; periosteal and endosteal diameters however showed no differences. Lastly, on assessing the effect of smoking on bone geometry, only EndoC and PeriC showed significant differences between the two groups. (103)

Cousins *et al.* (113) studied the association between muscle power and physical activity measured by Physical Activity Scale for the Elderly (PASE) with cortical bone geometry and strength at the 33% radius and 66% tibia in older men from The Osteoporotic Fractures in Men Study (MrOS). After adjustment for age, race, tibia length, and weight: SSI, SM, and CA increased across quartiles of leg power at the tibia and were significant for trend (p-value for SSI = 0.001; p-value for SM < 0.001; p-value for CA = 0.003). Similar associations were observed for physical activity (p-value for SSI = 0.014; p-value for SM = 0.006; p-value for CA = 0.006). At the radius, SSI, SM, TA, and CA increased across quartiles of grip strength and physical activity and were significant for trend (p-value for all < 0.001). Overall, differences of 6% and 13% were found between the lowest and highest quartiles of physical activity and grip strength respectively. At the tibia, SSI was 5% greater, while SM was 6% greater when comparing the lowest to the highest quartile of leg power.

2.7.2.2 Endocrine factors

When examining the effect of the relationship between bioavailable sex hormones and bone structure in men from Manchester and Leuven participating in the EMAS, Ward *et al.* (104) found no significant associations except a negative relation with TA and bioavailable estradiol

(E2) in Manchester men. Among Leuven men, CrtThk showed a positive association with E2, whereas MA showed a negative association. Bioavailable testosterone (T) did not show any significant associations with measures of bone structure. These associations persisted after adjusting for age, weight and height.

Khosla and colleagues (114) also examined the relationship between bioavailable sex hormones and bone geometry in 325 men aged 22 – 93 men from the Rochester Epidemiology Project. In young and middle-aged men, they did not find any association between bone geometry and bioavailable T and E2, except an inverse association between CA at the distal tibia with bioavailable T in young men. In elderly men, TA, MA were inversely associated with E2 and T at the distal tibia. Furthermore, CA at the femoral neck was positively associated with bioavailable E2 levels.

2.7.2.3 Medical Conditions

Type 2 diabetics have increased fracture risk (115,116), despite higher BMD. (117–122) This paradoxical relationship is only partially explained by the increased propensity to falls (123,124) due to complications of type 2 diabetes (T2D). (125–127) Hyperglycemia in T2D leads to the accumulation of advanced glycation end products bringing about glycation of the bone matrix rendering it brittle. (128) Moreover, T2D disproportionately affects those of African ancestry. (129) There is scant information on the relationship of T2D and bone geometry and bone strength in black men. In middle-aged Afro-Caribbean men, having a history of T2D was independently related to gains in cortical BMD at the radius and tibia. (130) Recent cross-sectional analyses of T2D and appendicular bone geometry and strength in a cohort of older Caucasian men found enhanced trabecular BMD and compromised cortical bone strength due to smaller TA. (120,131) The smaller PeriC among type 2 diabetics (131) contrasted with evidence from animal

studies (132,133) highlighting the need for further study as: cortical bone constitutes 80% of the skeleton, and in T2D most fractures occur at cortical sites. (117,119,121,122)

3.0 Osteoporosis

Osteoporosis is an asymptomatic systemic bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. (86) Despite posing a serious health problem and a considerable economic burden, osteoporosis is widely overlooked especially in men. One in eight men over the age of 50 will experience an osteoporosis-related fracture in their lifetime. (134) It has been shown that the morbidity and mortality after fractures is greater in men than women. (135–137) As the world population ages and longevity improves, a better understanding of male osteoporosis is vital as osteoporosis-related fractures are preventable.

3.1 Fracture Epidemiology in Men

Fracture incidence in men shows a bimodal distribution: those occurring in young adulthood are likely of traumatic origin; while those occurring in late life after age 70 have been attributed to increased propensity to falls and increased bone fragility. (138) Based on age-specific incidence of fractures in over 100,000 men registered in General Practice Research Database in the UK, the incidence of fractures at the femur, vertebrae, radius/ulna, humerus, clavicle, scapula, ribs, and pelvis increased with aging in men. Therefore, fractures at these sites were considered more likely to be associated with osteoporosis, unlike fractures of the skull, hands, feet, and distal lower extremities, that did not show an increase with aging in men. (138) In the Rotterdam Study, the proximal femur, forearm and, humerus were the most common sites for fracture in men aged

55 years (yrs) and over. (82) Similar trends were reported in predominantly white men from the United States. (139) In Australian men aged 60 and over, Center *et al.* demonstrated that after initial fracture the relative risk for subsequent low-trauma fracture sustained by falling from a standing height was 3.47 (RR 3.47; 95% CI, 2.68 – 4.48). (140) An analysis of data from MrOS showed that even high-trauma fractures were also associated with low BMD in older men; for every 1 standard deviation (SD) reduction in BMD, the multivariate risk hazard was 54% higher for high trauma fractures (95% CI, 1.20 – 1.96) and 69% higher for low-trauma fractures (95% CI; 1.49 – 1.91). (141)

3.1.1 Hip fractures

While hip fractures are rare in men under 75 yrs of age, the risk increases exponentially after the 75th year. Even though the absolute incidence of hip fractures shows geographic variation, (142) the increased hip fracture incidence in elderly men has been observed globally: in North America, (143–146) Europe, (82,138,147–160) Asia, (151,161–165) Australia, (166–168) South America, (151,169), and Africa. (170,171) Men from Scandinavian countries, other Northern European countries and white men from North America had the highest incidence of hip fractures, (172,173) while black and Asian men had the lowest incidence. (172,173) Low rates were also reported in men from South America and equatorial regions. (142,172,173) Every 10° increase in latitude corresponded to a 0.3% increase ($p < 0.001$) in the 10-yr probability of hip fracture in men, even after adjusting for economic prosperity. (174)

Following a hip fracture, men are twice as likely as women to die in the hospital. (137,175) Also, the 1-yr mortality rate ranges from 31 – 35% in comparison to the 17 – 22% observed in women. (136,176–178) These differences in rates of mortality is explained by the higher number

of comorbid conditions in men. (137,179–181) Further, almost half the men suffering from hip fractures require institutionalized care (175,181) and many who return home after hip fracture are unable to regain pre-fracture levels of function. (181) As such, men are less likely than women to be living independently 1-yr after hip fracture. (176) Following a hip fracture, the decrease in life expectancy for men aged 60 – 69 yrs is 11.5 yrs; for those aged 70 – 79 yrs, the decrease in life expectancy is 5 yrs; lastly, for those aged 80 and above the decrease in life expectancy is 1.5 yrs. (135)

3.1.2 Vertebral fractures

Estimating the burden of vertebral fractures poses a challenge because vertebral fractures are often asymptomatic and many are noted as incidental findings on radiographs. However, recording these asymptomatic vertebral deformities is important and clinically relevant because they are associated with low BMD and increased risk for osteoporotic fracture.

The European Vertebral Osteoporosis Study (EVOS) estimated the prevalence of vertebral deformity assessed by radiographs in 15,570 men and women aged 50 – 79 from 19 European countries. Depending on the defining criteria used, the prevalence was 12 or 20% in either sex, and showed an age-related increase. (180) Similar trends were reported in a US Study of men aged 50 and over (182) and a Finnish study of men aged 40 and over. (183)

As part of the European Prospective Osteoporosis Study (EPOS), 14,011 men and women aged 50 and over from population-based registers in 29 European countries were followed for an average duration of 3.8 yrs. (184) In men the age-standardized incidence of vertebral fractures was 5.7 per 1000-person yrs at risk using morphometric criteria and 6.8 per 1000 person yrs at risk using qualitative assessment. Other groups have found the age-adjusted incidence of vertebral

fractures in men to be half the rate found in women. (185,186) Similar to hip fractures, the incidence of vertebral fractures increases with age. (138,145,166,167,184) Moreover, geographic variations in incidence of vertebral fractures are also observed with the highest rates seen in Sweden. (184)

Vertebral fractures predict subsequent fractures. (187–190) Furthermore, in the Swedish cohort of EVOS, prevalent vertebral deformity predicted mortality in the forthcoming decade (HR: 2.4; 95% CI: 1.6, 3.9). (190) Moreover, severe vertebral deformity was associated with functional impairment (191) and negative outcomes in men. (192)

3.1.3 Other fractures

Non-spine and non-hip fractures contribute to significant morbidity and healthcare costs in men and women; even though they are not commonly described, they are indicative of low BMD and are associated with fractures at other sites. (193)

In men, the incidence of fractures at the distal radius is stable through life with a slight increase at older ages. (147,194–196) However, reports suggest an elevated risk of hip and vertebral fractures following distal forearm fractures. (197,198)

Fractures of the proximal humerus are common in men (145,147,157,158,167) and associated with greater 5-year mortality as compared to women. (199) Their incidence appears to be increasing over time. (157) Moreover, low trauma fractures of the upper limb increase the risk of subsequent fracture. (140) Pelvic fractures increase with aging in men and are associated with osteoporosis. (200,201) Fractures of the femoral and tibial shaft also show a bimodal distribution with higher incidence in men aged 15 – 34 and those over 70. (147) Though not traditionally osteoporotic, these types of fractures have been shown to be associated with elevated risk of

subsequent fracture in men. (140) Projections from three decades of fracture information in an elderly Finnish population suggest low-trauma fractures also increase with age. (202)

By the year 2050, projections for hip fractures alone in elderly men range from 1.8 million (143) to 6.3 million. (203) The higher morbidity and mortality in men compared to women following hip fractures is well established. (135–137,175–178,204–208) Despite these reports, men continue to be undertreated for osteoporosis after hip fractures (177): a mere 7% men received any treatment at discharge as compared to 31% women; at 1 – 5 yr follow-up 27% men received Vitamin D or calcium in comparison to 71% women. While the epidemiology of fractures has been described in diverse populations, Caucasian men have been studied in greater detail particularly compared to men of African ancestry.

3.2 Bone Health in African Ancestry Men

There is a paucity of information on QCT-assessed bone quality and its determinants in African ancestry men. Skeletal health along with its determinants in black men have been studied in the following studies: the Baltimore Men's Osteoporosis Study, (209) Health, Aging, and Body Composition Study (Health ABC), (210) MrOS, (211,212) the Boston Area Community Health/Bone study, (213,214) and the Tobago Bone Health Study. (215)

3.2.1 Studies of areal BMD and correlates

In one of the first studies to longitudinally assess racial differences in rates of bone loss in older men aged 65 and over, both absolute and percentage declines in BMD at the femoral neck

and total hip were higher among white men. (216) The rate of decline at the femoral neck in black men was $1.1 \pm 3.3\%/yr$ and approximately $2.1 \pm 3.7\%/yr$ for white men ($p = 0.01$). At the hip, BMD remained unchanged for black men unlike white men who lost $0.8 \pm 2.8\%/yr$ ($p=0.007$). However, the differences disappeared upon adjustment for age, weight, weight change, smoking and baseline BMD. White race, old age, current smoking, and low baseline BMD were associated with greater rate of decline of BMD at femoral neck and hip. Low baseline weight was also associated with greater decline at the femoral neck. Change in weight between visits was not related to rate of decline. Old age and smoking remained significant independent predictors of rate of decline at femoral neck. No relationship was observed with alcohol consumption and physical activity. Tracy *et al.* (216) also reported bone mineral apparent density (BMAD) that declined at a rate of $1.9 \pm 4.6\%/yr$ among black men and $3.6 \pm 7.9\%/yr$ among white men ($p = 0.03$). White race, old age, and baseline BMAD were associated with greater decline in BMAD. Unlike for aBMD, the race differences in BMAD persisted upon adjustment for age, baseline weight, weight change, current smoking, and baseline femoral neck BMAD. In the multivariable model, age, lower baseline weight, and baseline femoral neck were independent predictors.

Using a cross-sectional design, Taaffe and colleagues examined race differences in aBMD and its determinants in 544 black elderly men aged 70 – 79, from the Health ABC study. (217,218) Lean mass was an independent predictor of BMD at femoral neck, upper limb, lower limb and whole body respectively. One SD increase in lean mass corresponded to 5.9%, 3.1%, 3.5%, and 3.7% increases at BMD at femoral neck, upper limb, lower limb and whole body in models adjusted for age, site, height, lifestyle and thiazide usage. (217) However, there was no independent association between femoral neck BMD and measures of physical function assessed by knee extensor strength, chair-rise capacity, usual gait speed, and balance. Unlike femoral neck

BMD, BMD at the trochanter increased across quartiles of knee extensor strength and chair-stand performance and was positive for linear trend ($p = 0.007$ and $p = 0.041$ respectively) after adjustment for age, study site, height, weight, smoking, medications and physical activity. (218)

In a study combining data from MrOS and the Tobago Bone Health study, Sheu and colleagues (219) compared and reported annual percentage changes in DXA-measured hip BMD and femoral neck BMD, BMAD, cross-sectional area (CSA) and BMC in Caucasians, African-Americans, Asian Americans and Afro-Caribbeans. BMD, BMAD and BMC were highest among Afro-Caribbeans, followed by African Americans, Caucasians, and Asian Americans. CSA on the other hand was highest in Caucasians, followed by Asian Americans, African Americans, and lowest in Afro-Caribbeans. Differences between Caucasians and African ancestry men (African Americans and Afro-Caribbeans) remained significant after adjusting for age, weight, height, study site, history of fracture, history of prostate cancer, and diabetes. However, over a follow-up period of 4.5 yrs, Caucasian, African American, and Afro-Caribbean men experienced BMD loss of 0.26 – 0.54%/ year. Rates of change in CSA were not reported.

Travison and colleagues studied the association of lean and fat mass with aBMD and DXA-measured bone strength at the proximal femur in 1171 white, black and Hispanic men aged 30 – 79 yrs from the Boston Area Community Health/Bone study. (220) They found that the positive relationship between a 10-kg increase in fat mass and BMD and bone strength was reversed or disappeared after adjusting for lean mass. On the contrary, a 10-kg increase in lean mass was positively associated with BMD and bone strength even after adjusting for fat mass. Upon adjusting for age, height, fat mass and lean mass in comparison to white men, black men had BMD that was 8.6% higher (95% CI: 5.8, 11.4); CSA that was 5% higher (95% CI: 2.3, 7.6);

average buckling ratio was 13.6% lower (95% CI: -17.8, -9.4); and bending strength that did not show any significant differences.

In a population-based sample of middle-aged men from the Tobago Bone Health Study, Hill *et al.* described aBMD and its determinants using a cross-sectional design. (221) For every 10-year increase in age, aBMD decreased by 2.5% at the hip and 4% at the femoral neck. Men who self-reported mixed ancestry (having one or two African ancestry grandparents) had 6 – 7.5% lower BMD at hip and femoral neck. Of the anthropometric factors, lean mass emerged as the strongest correlate: it was associated with 6 – 6.5% higher age-adjusted aBMD for every SD increase in lean mass. Similarly, one SD higher body weight was associated with 5.4% higher age-adjusted aBMD. Lastly, one SD change in height and grip strength were associated with a 2% increase in age-adjusted aBMD. Of the lifestyle factors evaluated: history of working on a farm or on a fishing boat was associated with 1 – 3% higher aBMD at hip and femoral neck. Smoking was not associated with BMD at either the hip or femoral neck. Hypertension and diabetes were associated with 3 – 4% higher aBMD. However, no medications showed any significant associations with aBMD. In multivariable analyses, a model including age, mixed ancestry, lean mass, lifestyle factors, diabetes and hypertension explained 25 and 27% of the total variance for hip and femoral neck aBMD respectively. However, lean mass alone explained 18 – 20% of the variation. Similar associations albeit of lower magnitude were observed with BMAD. The correlates for aBMD explained about 13% of the variance in BMAD. Overall, these findings suggest that ethnic differences in lean mass could potentially explain the differences in ethnic differences in fracture rates.

The cross-sectional findings reported by Hill and colleagues (221) were confirmed by Sheu and colleagues (222) in a longitudinal study. BMD decreased at a rate of $0.10 \pm 0.55\%/yr$ at the

hip and $0.29 \pm 0.81\%/yr$ at the femoral neck in middle-aged men whose average age was 56 yrs. Upon stratifying the men in 5-year age groups, a U-shaped relationship was observed at the hip and femoral neck: men aged 40 – 44 had a greater rate of BMD decline as compared to men aged 45 – 49 and 50 – 54 followed by accelerated loss after age 60. This relationship persisted after adjusting for important covariates like weight, weight change, hypertension, diabetes, arthritis, grip strength, and current smoking. A 10-kg increase in body weight was associated with a slower rate of decline. Other measures of body composition like total lean mass, whole body fat and total body fat percentage showed similar associations. Initial BMD was not related to rate of decline. Men who lost weight during follow-up had significantly greater rate of aBMD decline. Every 6% decrease in body weight was associated with an increased rate of loss by a magnitude of $0.11\%/yr$ at the hip and $0.16\%/yr$ at the femoral neck. Lifestyle characteristics like smoking, alcohol consumption, and TV-watching (a measure of physical inactivity) were not related to rate of loss. Diabetes was associated with a greater rate of decline at the hip, but not at the femoral neck. At the hip and femoral neck, those who lost 5% or more of body weight had significantly greater decline in BMD. The effect of weight loss on rate of decline was pronounced in men older than 55 yrs of the age at both hip and femoral neck. Similarly, men with low BMI lost more bone irrespective of weight gain at the femoral neck. Age, BMI, weight change and grip strength emerged as independent correlates in multivariable models. The models explained about 5 – 6% of the variance in the rate of decline in BMD. The findings suggest early losses of femoral BMD and non-linear loss across age groups. (222)

3.2.2 Studies of volumetric BMD and correlates

Mackey *et al.* (223) examined the association of trabecular BMD and hip aBMD with the risk of non-vertebral fractures. The age-adjusted hazard ratio (HR) was highest for black men: hip aBMD: HR = 2.04, (95% CI:1.03, 4.04); vertebral trabecular BMD: HR = 3.00, (95% CI:1.29, 7.00). Although they did not report the HRs for black men separately, in models adjusted for age, sex, and race, one SD decrease in hip aBMD was associated with a 67% greater risk (95% CI: 1.36, 2.07), and one SD decrease in vertebral trabecular BMD was associated with a 47% (95% CI = 1.18, 1.82) greater risk.

Sheu *et al.* carried out a longitudinal study of middle-aged Afro-Caribbean men and reported losses at the trabecular and cortical compartments at radius approximating $0.047 \pm 0.767\%/yr$ ($p = 0.016$) and $0.254 \pm 0.222\%/yr$ ($p < 0.0001$), respectively. At the tibia, the rates of change were $0.006 \pm 0.707\%/yr$ ($p = 0.76$) and $-0.264 \pm 0.198\%/yr$ ($p < 0.0001$) for the trabecular and cortical compartments respectively. Unlike cortical BMD which showed statistically significant declines in all age-groups at both skeletal sites, trabecular BMD declined significantly at the radius in younger men aged 40 – 49 yrs. (130) Greater body weight at baseline was associated with increase in trabecular BMD at both skeletal sites. Weight loss during follow-up was associated with decreased trabecular BMD at the tibia but not radius. Additional independent correlates associated with trabecular bone loss at the radius included walking in the past seven days and use of proton-pump inhibitors, as well as hypertension and prostate cancer at the tibial site. Cortical bone loss at the radius and tibia shared several independent correlates, including diabetes, hypertension, active use of Androgen Deprivation Therapy (ADT), and having ever smoked. Weight gain was related to cortical bone loss at the radius. At the tibia, mixed race,

greater body weight, and greater grip strength were associated with an increase in cortical bone. Congestive heart failure was independently associated with cortical bone loss.

Overall, some DXA studies have shown a relationship between race and rates of bone loss even after adjustment. Higher lean body mass was positively associated with BMD at different sites in elderly black men. In a pooled study comparing white and black men from the US to Afro-Caribbean men, adjusted baseline bone strength assessed by DXA was highest in Afro-Caribbean men followed by African-American men and least in white men from the US. However, the rates of loss were similar across the three groups. Other studies of correlates of BMD suggested ethnic differences in body composition could potentially determine differing fracture rates. The sole study using QCT that focused on black men alone found the rates of bone loss and higher trabecular loss earlier in life to be comparable to white men.

3.3 Pathogenesis of Osteoporosis

3.3.1 Peak Bone Mass

The magnitude of the variance of bone mass and bone size about their age-specific mean is large; one SD is nearly 10 – 15% of the mean. The variance of the rate of bone loss is a whole order of magnitude lower, one SD is about 1% of the mean. (224) These two observations underscore the importance of PBM which is acquired by the 3rd decade, as a crucial determinant of the lifetime risk of osteoporosis. (224,225) By 18 yrs of age, men and women acquire 90% of their PBM. (225–228) Gains of approximately 5 – 12% in bone mineral density have been reported in the 3rd decade of life. (225,229)

Over 50% of the variability in peak bone mass is due to genetics. It is achieved by modeling and remodeling occurring at varying rates at various skeletal sites. (225,230) Genome-wide association studies have suggested there might be genetic variants relevant only to male osteoporosis by affecting testosterone regulation. (231) During puberty, sex steroids, GH/IGF-1 control bone accrual. (232) The timing of puberty is also important in determining the acquisition of peak bone mass. (233) High levels of free T were a positive predictor of cortical bone size, whereas high levels of free E2 were a negative predictor of bone size. (234) During puberty, boys achieve greater bone mass, grow taller, and have greater periosteal apposition as compared to girls. These characteristics result in greater bone strength among boys as compared to girls even though they both have similar vBMD. (235,236)

The variance in cortical BMD, which is a function of the degree of matrix mineralization and intracortical porosity, decreased in adolescence (237,238) suggesting that the bone's material composition may become similar in individuals as they mature; therefore, variance in bone strength may be more due to bone structural differences rather than material.

3.3.2 Age-related bone loss

While an acceleration of bone loss with advancing age has been noted from studies utilizing DXA, (32,33,222) the use of QCT has facilitated the study of the trabecular and cortical compartments separately and exposed the partial nature of existing theories of bone loss. (27)

In a study of 323 white men aged 20 – 97 yrs underwent central and peripheral QCT, (97) over the lifespan, nearly half of the trabecular BMD was lost in men at the lumbar spine and femoral neck ($p < 0.005$ for both). At the femoral neck, cortical BMD decreased by 13% ($p < 0.05$). At the distal radius, cortical BMD decreased by 21% and trabecular BMD by 27% ($p < 0.005$ for

both). At the distal tibia, similar rates were seen, trabecular BMD decreased by 25% and cortical by 22% ($p < 0.005$ for both). Annual loss of cortical BMD was approximately 0.5%/year. (97)

These findings were confirmed in a longitudinal study after 3 yrs of follow-up. (27) Loss of cortical BMD picked up after 75 yrs of age, while trabecular BMD losses began early in life and continued through old age. (27) The loss of cortical BMD likely reflected increased porosity; the authors suggested that as interstitial bone changes little with aging, (239) bone loss arises from increased cortical porosity due to increased number of resorption cavities (240,241) and incompletely closed osteons. (239) Cortical bone is lost due to increased endosteal resorption and by increase in porosity.

The findings of Russo and colleagues, (98) who reported linear declines in trabecular and cortical BMD at the 4% and 38% sites respectively in older Italian men, supports those of Riggs and colleagues. (97) As did the findings from a study of 807 Icelandic men aged 67 – 93 as part of the AGES – REYKJAVIK. (99) Trabecular BMD decreased at the lumbar spine at a rate of 14.9%/decade ($p < 0.0001$), while cortical BMD did not show significant changes. Similar magnitudes of change were noted at the femoral neck: trabecular BMD decreased 14.2%/decade ($p < 0.05$) and cortical BMD did not change significantly. At the trochanter, cortical BMD decreased by 1.1%/decade ($p < 0.05$). Furthermore, in a cohort of 620 Chinese men aged 20 – 98 (100), cortical BMD loss commenced at age 60 yrs. Loss of trabecular BMD started in early adulthood and showed a steady decline. Trabecular and cortical BMD losses occurred at the radius and tibia, except in older men at the tibia.

Together these studies showed that bone loss rates increased with age accompanied by an imbalance between bone formation and bone resorption, (242) compromising bone strength in the process as endosteal resorption overtakes periosteal apposition. (243)

3.3.3 Inflammation and skeletal health

Osteoporosis is a well-known consequence of inflammatory joint and bowel diseases. (244–248) Chronic low-grade inflammation is associated with the development of disorders of cognition, (249) cardiovascular disease, (250) and diabetes, (251) prompting an exploration of a relationship between low-grade inflammation and fracture risk.

Rodent studies and laboratory data suggest that IL6, interleukin-1 (IL1) and tumor necrosis factor-alpha (TNF α) play important roles in bone remodeling and the pathogenesis of osteoporosis. (252,253) Two physiological pathways potentially favoring osteoclastogenesis explain this relationship: Proinflammatory cytokines increase the expression of RANKL, decrease OPG and increase M-CSF in stromal cells (254); estrogen deficiency increases the rate of bone resorption by increasing osteoclast numbers. (255,256) It is noteworthy that other well-known risk factors for fractures, like smoking and low physical activity, are mediated through inflammation. (257–259) Thus, the overlapping pathways between bone biology and inflammation biology lend support to the idea that osteoporosis results partly from an inflammatory process and could potentially be considered an immune disease. (260–262)

3.3.3.1 Epidemiology of biomarkers of inflammation and skeletal health

The epidemiological evidence examining the link between inflammation and skeletal health comes largely from studies conducted in women. (263–266) Low-grade inflammation was assessed by measuring serum CRP or hsCRP in all studies and the outcome was fracture risk. A dose-response relationship was reported by Pasco and colleagues in Australian women with median age of 77 years. (263) In a cohort of Japanese women with mean age of 75 years, those in the highest tertile were at greater risk for fractures. (265) And Ishii and colleagues found an

increased risk of fracture at levels of hsCRP exceeding 3mg/L (266) in women aged 42 – 53 participating in the Study of Women's Health Across the Nation (SWAN).

Schett and colleagues (264) and Cauley and colleagues (267) studied mixed groups from the Bruneck Study aged 50 – 70 and Health ABC aged 70 – 79 respectively. In the former, there were too few fractures in men and the latter reported the results of the men and women combined and found a relationship of borderline significance when comparing the highest quartile of hsCRP to the other quartiles combined. The investigators did not find an association between BMD and hsCRP; similarly, the relationship with fractures was also independent of BMD.

Barbour and colleagues (268) assessed the relationship between hip fractures and serum levels of the soluble receptors (SR) of IL6 and TNF- α (TNF SR1 and TNF SR2) to address limitations posed by the transient nature of elevated IL6 and TNF α levels and evidence suggesting elevations of soluble receptor levels represented more severe inflammation. (269,270) They used a nested case-control design and examined 50 – 79 yr old women from the Women's Health Initiative (WHI) cohort. When comparing women in the highest quartile of inflammatory markers to those of the other three combined, the authors found that TNF SR2 increased the risk of hip fracture by 56% (95% CI: 1.09 – 2.022) after adjusting for known risk factors. When women were categorized according to the number of elevated inflammatory cytokines, those who had elevated levels of all three types of soluble receptors had a risk ratio of 2.76 (95% CI: 1.22 – 6.25); this relationship was also positive for linear trend (p-value for trend = 0.018). After examining a slew of mediators, the final model included number of falls, physical functioning, sex hormone-binding globulin (SHBG) levels, E2 levels, T levels, and cystatin-C levels. Overall, having all three cytokines elevated amounted to a risk approximately equal to 1 SD decline in BMD. (271)

Overall, few studies have examined the rate of change in BMD in association with levels of inflammatory markers (272) and only one study has examined the association between changes in serum levels of inflammatory markers and changes in BMD. (273) Ding *et al.* found baseline IL6, hsCRP and TNF α significantly predicted 3-year change in DXA-measured total body aBMD after adjustment for age, sex, anthropometry, and smoking in 168 older adults aged 50 – 79 from the Tasmania Older Adult Study. Furthermore, 3-year change in IL6 was a significant predictor of changes in aBMD at the hip and spine; similarly, TNF α was a significant predictor of changes in aBMD at the spine. Lastly, in models including all the markers of inflammation, IL6 emerged as the primary predictor of BMD. (273) In a sample of 137 postmenopausal German women from EPOS, Scheidt-Nave and colleagues reported a statistical interaction between menopausal age and IL6 levels: IL6 was the most important predictor of bone loss at the hip within 10 years of menopause and not beyond. A similar nonsignificant association was observed with bone loss at the spine. (272)

Some groups have studied only men (274,275) or carried out separate analyses in men, (264,276–278) while others have pooled men and women in the analyses. (267,273) One of the first studies, which examined the relationship between low-grade inflammation and bone health in men, used a sample of 500 Italian men from the Bruneck Study. (264) A total of 19 nontraumatic fractures occurred corresponding to an incidence rate of 3.4 per 1000 person-yrs. The investigators assessed the potential role of serum hsCRP as a risk factor for nontraumatic fractures. Firstly, they observed a dose-dependent relationship in the incidence of nontraumatic fractures across tertiles of hsCRP; the incidence was 1.3, 3.8, and 13.9 per 1000 person-years in tertile one, two and three respectively. Secondly, using pooled logistic regression, the adjusted Odds ratio (OR) for fracture in men comparing the highest to the lowest tertile was 13.2 (95% CI 1.3 - 126.7); moreover, the

ORs were significant for trend across tertiles ($p = 0.004$). One of the strengths of this study was the adjustment for known risk factors, Ultrasonogram (USG)-measured BMD, markers of bone metabolism like RANKL, OPG, renal function and medication use. Further, all medical conditions and fractures were validated. Additionally, the investigators assessed the robustness of their results by showing consistency on excluding various chronic conditions and using clinically-meaningful cut points of hsCRP. It is noteworthy that overall hsCRP was unrelated to BMD and inversely related to markers of bone metabolism; however, in a small subset of individuals with hsCRP $> 7.5\text{mg/L}$, there was an inverse relationship with aBMD and positive relationship with markers of bone metabolism. Overall, hsCRP was an independent predictor of nontraumatic fractures.

To elucidate the mechanism linking fractures and inflammation, de Pablo and colleagues used a nationally representative sample of 5261 men aged 20 and over to examine the relationship between aBMD and hsCRP. There was an inverse dose-dependence of BMD across quintiles of hsCRP in both limbs, which was significant for trend after adjustment with important risk factors including Vitamin D. The magnitude of the differences in BMD across quintiles of hsCRP was comparable to the differences in BMD across Vitamin D concentrations (279) or Vitamin D and calcium supplementation doses. (280)

As inflammatory markers predicted fracture independent of BMD in some studies, impairments in bone microarchitecture were hypothesized as the underlying mechanism. Rolland and colleagues (275) tested this hypothesis by evaluating the relationship between hsCRP and HR-pQCT-measured bone microarchitecture in 1149 men aged 19 – 87 years from the Structure of the Aging Men's Bones (STRAMBO) cohort. Based on the analysis of a LOESS curve, they categorized men into two groups: below and over the age of 72. In men younger than 72, bone microarchitecture not associated with hsCRP. In men over 72 after adjustment, hsCRP was

associated with unfavorable trabecular microarchitecture: lower trabecular BMD, lower trabecular number (TbN), more trabecular separation (TbSp), and more heterogeneous distribution. The relationship was consistent when examined across quartiles or clinically-meaningful categories. Furthermore, prevalent fractures showed a dose-response relationship with hsCRP. The OR for prevalence fracture was 2.22 (95% CI: 1.29 – 3.82) comparing men with hsCRP values >5mg/l to those with hsCRP < 1mg/l; unexpectedly, this association was not altered on adjustment for microarchitecture. Similar yet stronger relationships were noted in men over 72 years of age. In contrast, no association was seen with tibial measures or cortical microarchitecture. Overall, impairments in microarchitecture did not explain the association between hsCRP and fracture risk. Although, Ishii and colleagues did not assess bone microarchitecture, they reported hsCRP was negatively associated with bone strength and not BMD in a longitudinal study of women with a 10-year follow-up period. (266) They attributed these findings to inflammation-mediated disruption of the balance between bone strength and mechanical load.

The European Prospective Investigation of Cancer (EPIC)-Norfolk study of men aged 40 to 79 is one of the largest studies of the link between bone health and inflammation. (277) A U-shaped relationship was observed between all fractures and serum hsCRP. One of the limitations of this study was grouping traumatic and nontraumatic fractures, as traumatic fractures tend to occur at younger ages, and could potentially explain the U-shaped relationship. However, the men in this study were aged 40 and over and the U-shaped relation prevailed even after adjusting for common confounders and risk factors.

Turning the focus to older men, Eriksson and colleagues (274) examined the relationship between incident fractures and hsCRP in 69 to 81-year old men from the MrOS study in Sweden. When comparing the highest tertile of log transformed hsCRP with the lower two combined, the

adjusted risk for all fractures was 48% higher (HR 1.48, 95% CI 1.20 – 1.82). The risk was 61% higher for vertebral fractures (HR 1.61, 95% CI, 1.12 – 2.29). These relationships remained after adjustment for aBMD, suggesting that other mechanisms are at work. After excluding fractures within the first three years of follow-up, the relationships between hsCRP and incident fracture grew stronger for all fractures (HR 1.61, 95% CI 1.18 – 2.20) as well as vertebral fractures (HR 1.89, 95% CI 1.19 – 3.02). This augmentation of the association might suggest the effect of persistent low-grade inflammation.

Recently, Sponholtz and colleagues (278) used data from the Framingham Offspring Study to examine the cross-sectional relationship between inflammation and BMD 1293 middle-aged to older men. Unlike preceding studies, they used TNF α and IL6 and found no relationship between inflammatory markers and BMD after adjustment for height, weight, smoking and physical activity, leading them to suggest that the measurement of inflammatory markers may be of little utility.

3.4 Risk factors for low-BMD fractures in Men

With male osteoporosis gaining attention as a public health problem, a systematic assessment of risk factors for low bone mass-related fractures was carried out. Advancing age, low body mass index (BMI), family history of fractures, prior fracture, and lifestyle factors like current smoking and alcohol consumption increased the risk of fracture. With every one-year increase in age the odds of fracture increased 12% (OR = 1.12, 95% 1.07, 1.18). In men older than 70 as compared to those aged 70 and younger, the risk of fracture was 52% greater (95% CI: 1.11,2.08).

Low BMI was associated with an OR of 1.12 (95% CI: 1.04, 1.20). For every 1 SD decrease in BMI, the OR was 1.30 (1.15, 1.47).

Underweight has been a consistent risk factor for fractures, (281) while the association with overweight and obesity hadn't been studied much. In a sample of older men, most hip fractures occurred in obese men. The associations although attenuated persisted after adjustment for BMD and falls. (282) Black race was protective with an OR of 0.69 (95% CI: 0.57, 0.85). Daily consumption of alcohol or consuming more than 10 drinks per week was associated with 28% higher odds (95% CI: 1.08, 1.53). Current smoking was associated with a nearly 50% higher odds of fracture (95% CI: 1.29, 1.72). Other factors included prior fracture, family history of fractures, history of falls and chronic diseases like diabetes, asthma, cardiovascular disease (CVD), etc.

3.4.1 Epidemiological link between adiposity and skeletal health

Body weight (283–288) and lean mass (289–292) are among the strongest correlates of DXA measures of BMD in cross-sectional studies of men. Other studies suggest that fat mass and obesity may have a negative association with DXA measures of BMD in men. (289,293,294) Cross-sectional studies have shown a consistent relationship between higher body weight and BMD across studies in different geographical region. (295–298) For every 10kg increase in weight, BMD was 3 – 7% higher. Longitudinal studies have also shown weight loss to significantly increase the risk of low BMD at the hip in univariable and multivariable models. (30,31,299,300) Men who lost 5% or more of their body weight had doubled their rates of bone loss as compared to those who maintained their weight in the Framingham Study. (30)

Epidemiologic studies, due to limited sample size, have not been able to test the effect of fat mass independent of body weight. Hsu and colleagues addressed this limitation in a study of

7137 Chinese men aged 25 – 64 years. (301) Using DXA-measured BMC and BMD, Hsu *et al.* examined the relationship between bone mass and quartiles of percentage fat mass across seven 5kg-weight strata. They reported negative associations in every stratum at the hip for BMC. A significant linear trend for higher ORs of non-spine fractures, osteoporosis and osteopenia was found with higher percentage fat mass (%FM) in models adjusted for age, physical activity, occupation, smoking, alcohol consumption, weight, height. In men, the OR for osteoporosis comparing the lowest to highest quartile of %FM was 5.2 (95% CI: 2.1 – 13.2).

Zhao and colleagues (302) examined the relationship of obesity and bone mass excluding the mechanical loading effects of body weight. They used DXA-measures of bone mass and fat mass from two large samples of Caucasian and Chinese men and women having average ages of 62 and 27 respectively. Furthermore, they also examined the genetic and environmental correlations — correlation due to shared genes and environmental factors respectively — between obesity and bone mass. Overall, Zhao *et al.* reported that fat mass by itself did not have a protective effect; and shared environmental and genetic factors may help in reducing the burden of both obesity and osteoporosis. In both Chinese and Caucasian subjects, BMI and weight were positively correlated with bone mass. On examining the association between fat mass and weight-adjusted bone mass, an inverse relationship emerged. The association of lean mass and weight-adjusted bone mass was positive, suggestive of mechanisms other than mechanical loading. In contrast, the association between fat mass and weight-adjusted bone mass was negative suggesting that fat mass does not increase bone mass when mechanical loading due to weight has been accounted for. Weight-adjusted bone mass and lean mass showed significant positive genetic and environmental correlations. In contrast, genetic and environmental correlations of weight-adjusted bone mass and fat mass were significantly negative.

Travison and colleagues studied the association of lean and fat mass with aBMD and DXA-measured bone strength at the proximal femur in 1171 white, black and Hispanic men aged 30 – 79 years from the Boston Area Community Health/Bone study. (220) They found that the positive relationship between a 10-kg increase in fat mass and BMD and bone strength was reversed or disappeared after adjusting for lean mass. On the contrary, a 10-kg increase in lean mass was positively associated with BMD and bone strength even after adjusting for fat mass.

Overcoming the limitations of DXA in capturing both bone and adipose tissue compartments, Gilsanz and colleagues used QCT-measured femoral strength, regional adipose tissue, and muscle area in a sample of 100 young women aged 15 – 25 years. The basis for the study was the adverse metabolic profile associated with VAT as compared to subcutaneous adipose tissue SAT. They reported that VAT and SAT had negative and positive associations respectively with femoral bone strength after adjustments for limb length and thigh musculature. (303) Multiple linear regression models did not include weight as a surrogate for mechanical loading, instead muscle area was used.

Yerges-Armstrong *et al.* (304) further extended the research to regional adipose tissue depots and BMD by studying the cross-sectional association of calf fat and vBMD in a sample of Afro-Caribbean men aged 40 and over. They reported higher TF at the calf was associated with lower cortical BMD, an association that persisted on adjustment with age, height, muscle cross-sectional area at the calf, weight, and lifestyle factors. Sensitivity analyses showed that the associations remained the same in a subsample of only non-diabetic men, as well as, after adjusting for bioavailable T in a subset of men.

Extending the research to include bone quality, Ng and colleagues (305) examined a variety of bone quality measures at clinically-significant sites such as the lumbar spine (LS), femoral neck

(FN), and ultra-distal radius (UDR) using a combination of precise imaging methods like DXA, QCT and high Resolution pQCT (HR-pQCT). They studied mostly white men and women of ages ranging from 21 – 97 years. In older men, positive but nonsignificant correlations were seen with total body fat (TBF) and aBMD, total vBMD, trabecular and cortical BMD at all bone sites as well as trabecular microstructure at the UDR. Significant positive correlations were reported between TBF, SAT and VAT with cortical thickness at FN, LS, CA and TbN at the UDR. All these positive relationships were lost upon adjustment for age, bioavailable E2, height and particularly weight. Their observations in young men were the most disconcerting. In younger men, VAT was negatively associated with BMD at all three skeletal sites. Similarly, negative relations were seen with CrtThk and trabecular microarchitecture at UDR. Adjustment with age, height, bioavailable E2, and weight did not alter the relationship between VAT and bone measures. In contrast, the negative relation was accentuated at the UDR for CA. Overall, their results suggest that for any given weight, greater fat mass is detrimental to bone quality. As there is no difference between weight-bearing and non-weight-bearing sites, the mechanism involved is likely something other than biomechanical loading.

4.0 Knowledge Gaps

The majority of the bone geometry studies are limited by their cross-sectional nature. (98–100,103,104,114,306) Rates of change in bone geometry have been inferred by calculating differences in bone geometry and strength between different groups of individuals of extreme ages. (26,98–100) Moreover, only few studies have adjusted for stature, (26,99) which is related to bone size, yielding biased results due to changes in secular trends in height over the last century. Few data are available regarding rates of change in bone strength given by section modulus; the existing reports are contrasting: one group reported no change in CSMI in men, (98,107) while the other group reported a decline of 2%/decade. (100,105) The aging appendicular skeleton maintains bone's biomechanical competence by offsetting endosteal bone loss with periosteal apposition. However, not many have studied the patterns of age-related changes at these envelopes and their determinants. (103,104,113,114) While, Yuen and colleagues and Kaji and colleagues have shed light on the magnitude and some determinants of periosteal and endosteal circumferences in men of different ethnic groups, similar studies in black men are conspicuous by their absence. Lauretani and colleagues (106) reported that cross-sectional studies have underestimated rates of bone loss based on reports at the tibia highlighting the need to further study changes in bone geometry at various sites, to clarify the rates of periosteal expansion and endosteal expansion and assess their contributions to age-related bone loss in diverse populations.

The epidemiological evidence examining the link between biomarkers of inflammation and skeletal health comes from studies mostly focused on Caucasians in both sexes. (263–268,273–277,307–309) For biomarkers of inflammation to be of use in predicting fractures or to gain a deeper understanding of the etiology of osteoporosis, it is vital to study different population

groups. The data have been inconsistent for the link between aBMD and biomarkers of inflammation: some have found an independent relationship (263–265,267); others have reported an inverse relationship (273,307); and, yet, some others have found no significant relationship. (310–312) One potential explanation for this discrepancy is the limitation of DXA in capturing bone quality. Only two groups have addressed this limitation, Ishii and colleagues (266) and Rolland and colleagues (275) have examined DXA-measured bone strength and HR-pQCT-measured bone microarchitecture respectively. There is some debate regarding the utility of a single measure of IL6 in bone research, (313) while others have found IL6 to be the most important predictor of bone loss. (273) Some studies have assessed more than one inflammation biomarker (267,268,273,278) and only one has assessed the relationship between annual losses of BMD and changes in levels of inflammation markers. (273) It has been suggested that chronic inflammation might be more relevant in increasing the rate of bone loss necessitating more longitudinal studies examining this relationship. (272,273)

There is mounting evidence that adipose tissue compartments have differing effects on the risk of disease. (314–317) VAT in the abdomen has been examined, (303,305) but only Yerges-Armstrong and colleagues (304) have assessed fat in the subcutaneous and non-subcutaneous compartments of the calf. Most studies examining bone structure have been cross-sectional by design and conducted in young adults or adolescents. (294,318) The majority of the longitudinal work has been carried out in children with respect to growth and PBM attainment. (303,319–322) Few studies have used QCT to assess the relationship of adipose tissue on distinct bone compartments or bone structure and strength. (303,305) Furthermore, only one study has assessed the cross-sectional relationship between ectopic fat depots and cortical bone in young adults (323) and no study assessing relationships between changes in bone structure and fat deposits in men.

(324) While men of different ethnicities have been studied, only one study had sufficient numbers of African-American men to test the associations by racial group. (220) In summary, men, particularly men of African ancestry have been under-represented in studies of bone loss in general.

5.0 Rates and Correlates of Cortical Bone Structure and Strength Changes with Aging in African Ancestry Men

5.1 Abstract

As the population ages, more non-white men will develop osteoporosis and its associated fractures. However, very little is known about skeletal aging in men of African ancestry. To address this knowledge gap, we examined the rate of change in cortical bone structure and biomechanical indices of bone strength in 1576 African ancestry men aged ≥ 40 years (56.8 ± 9.1 years) during an average follow-up of 6.2 years. We examined changes in cortical thickness, periosteal and endosteal circumferences, and strength strain index, a measure of bone's torsional strength, using quantitative computed tomography at the radius and tibia. At both limbs, periosteal circumference increased at 2% per decade ($p < 0.001$) whereas endosteal circumference increased at a rate of 9 – 10% per decade ($p < 0.001$) such that there was a significant thinning of the cortices and decrease in SSI ($p < 0.001$). Anthropometric, lifestyle and medical correlates of these changes were identified and varied with skeletal site and measure examined. We conclude that endosteal resorption outpaces periosteal apposition with advancing age such that there is a net loss of cortical bone and reduction in bone strength in African ancestry men. Additional research is needed to better understand the biological factors associated with and clinical consequences of skeletal aging among men of African heritage.

5.2 Introduction

Osteoporosis is an asymptomatic condition characterized by low bone mass and deterioration of skeletal microarchitecture leading to increased risk of fracture. (325) Traditionally considered a condition afflicting predominantly woman, osteoporosis in men has gained increasing attention as a major public health issue due to the aging of the population. For example, there were an estimated 9 million osteoporotic fractures in the year 2000, approximately 40% of these occurred in men. (4) Moreover, hip and vertebral fractures in men are associated with higher morbidity and mortality than in women. (4,326)

Most longitudinal studies of bone loss and osteoporosis in men have focused on Caucasians. (30–36) Although men of African ancestry have higher peak BMD (37,38) and a lower prevalence of osteoporosis compared to men of other ethnicities, (327,328) rates of decline in BMD with aging appears to be comparable in Caucasian and African ancestry men. (39) Moreover, osteoporosis in African ancestry men is substantially underdiagnosed, undertreated and underreported. (329–332) Compounding the problem, as the population ages considerably more African ancestry men will suffer from osteoporosis (333,334) and there remains an urgent need to better understand skeletal health in African ancestry men so that high-risk men can be better identified for prevention and treatment.

Measurement of aBMD by DXA is the clinical gold standard; however, it is limited in its ability to capture bone microarchitecture or distinguish the trabecular and cortical bone compartments. QCT on the other hand measures vBMD, bone geometry and bone strength. Although BMD is a good surrogate for bone strength, bone fragility is also affected by the amount of bone tissue and its distribution within the bone envelope. The most efficient adaptation by the skeleton to resist bending forces is by distributing bone away from the center of the bone, a

property described as the cross-sectional moment of inertia (CSMI). The CSMI is proportional to the fourth power of the diameter of bone in cross-section, therefore, small increases in diameter lead to large increases in resistance to bending. The aging appendicular skeleton is characterized by this redistribution of bone material to preserve bone strength in the face of age-related loss of bone mineral density. (77)

In the present study, we examined age-related patterns and correlates of changes in cortical bone geometry (cortical thickness, periosteal and endosteal circumferences) and biomechanical indices of cortical bone strength (SSI) at the distal radius and tibia over an average of 6.2 years in the same men.

5.3 Methods

5.3.1 Study population

Between 1997 and 2003, 3,170 men aged 40 and older were recruited for population-based prostate cancer screening for the first time on the island of Tobago, Trinidad & Tobago. (335) To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the initial screening was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island parishes. The recruited cohort was 97% African, 2% East Indian, <1% white, and <1% "other" as defined by participant-report of paternal and maternal grandparents' ethnicity. The low non-

African admixture in this population (6%) has been confirmed using ancestry informative genetic markers. (336)

Between 2004 and 2007, men in the original cohort were invited to complete a peripheral QCT (pQCT) scan as part of the Tobago Bone Health Study. An additional 451 new participants were also recruited using similar methods and enrollment criteria as the initial screening study. A total of 2174 men underwent pQCT scans of the radius and tibia at this exam. (337) Between 2010 and 2013, we invited these men to return for repeat pQCT scans. Both the baseline and follow-up visits followed the same procedures for questionnaire interviews, biospecimen collection, pQCT and DXA scans. (337) A total of 1605 men completed the follow-up pQCT exam (80% of survivors). For the current analysis, we excluded men who were not of African ancestry yielding an analytical cohort of 1,576. Men who did not return ($n = 577$) were significantly older, lighter, shorter and had low grip strength. Men who returned had significantly or nearly significantly greater age-adjusted total bone area, cortical area, cross-sectional moment of inertia, section modulus and strength strain index as well as thicker cortices and larger periosteal and endosteal diameters at the tibia. At the radius, total bone area and the periosteal and endosteal diameters were not different between men who returned and those who did not (data not shown). The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study and all participants provided written informed consent before data collection.

5.3.2 Peripheral QCT

pQCT scans were performed at the non-dominant forearm and left tibia using the Stratec XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany). Technicians followed a

standardized protocol for patient positioning and scanning at both the baseline and follow-up visits. A scout view was initially obtained prior to the baseline pQCT scan to define an anatomic reference line for the relative location of the subsequent scans at the radius and tibia. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process. Scans were taken at 33% of the total length of forearm and tibia. Measurements at the 33% sites represent predominantly cortical bone. A single axial slice of 2.5mm thickness with a voxel size of 0.5 mm and a speed of 20 mm/s was taken at all locations. Image processing was performed by a single investigator using the Stratec software package (Version 5.5E). All 33% radius and tibia scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm³; peel mode 2; Cortmode 1) to determine bone geometric properties. SSI was determined with Cortmode 1 (T = 280mg/cm³). Cortical thickness (mm), periosteal circumference (mm), endosteal circumference (mm) and polar SSI (SSI, mm³) were measured at both radius and tibia. Coefficients of variation were determined for pQCT scans by replicating measurements on 15 subjects with CV \leq 2.1% for all measures. Daily phantom scans were analyzed to ensure long-term scanner stability. It is not possible to estimate material properties like elastic modulus from QCT measurements. However, vBMD measured by QCT has an approximately linear relationship with elastic modulus. Based on this relationship, Schiessl *et al.* (338) developed the SSI, which is the integrated product of section modulus and vBMD normalized to the maximal physiological cortical vBMD of human bones. Section modulus (mm³) was first calculated as $(a \times d^2)/d_{max}$, where a is the cross-sectional area of a voxel (mm²), d is the distance of the voxel from the center of gravity (mm), and d_{max} is the maximum distance or eccentricity of any voxel to the center of

gravity (mm). The ratio of cortical vBMD and normal physiological vBMD (1200 mg/mm³) provides an estimate of the modulus of elasticity.

5.3.3 Anthropometric measurements

Body weight was measured in kilograms with participants wearing light clothing and without shoes using a calibrated balance beam scale. Height was measured in centimeters without participants wearing shoes using a wall-mounted height board. Two height measurements were made and the average used in analysis. Waist circumference was measured at the umbilicus with an inelastic tape measure. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Handgrip strength was measured in kilograms for both the left and right hands as a surrogate for upper body and overall strength using a dynamometer (Preston Grip Dynamometer, JA Preston 136 Crop.). Average grip strength was based on two repeated measurements from left and right hands.

5.3.3.1 Other measurements

Trained interviewers and nurses administered questionnaires to participants. We focused on potential correlates of bone strength and bone geometry based on the literature and from our previous findings of correlates for BMD in this cohort. (339,340) We collected information pertaining to demographic characteristics, medical history, medication use, personal and family fracture history, physical activity, and lifestyle habits. Ethnicity was self-reported and participants provided detailed information on the ethnic origin of their parents and grandparents.

Participants were asked if they had been diagnosed with cardiovascular diseases, diabetes, respiratory diseases, hypertension, cancer and fractures. Family history of fracture was also

ascertained. Personal history of diabetes was defined as having a history of using diabetes medications or a fasting glucose level of 126mg/dL and above. Hypertensive men were defined as having a systolic blood pressure of 140mmHg or above, diastolic blood pressure of 90mmHg or above, or use of anti-hypertensive medication. Men were asked to report their history of selected medications as well as current medication use. Medication use was coded based on the Anatomical Therapeutic and Chemical and Defined Daily Dose system. (341) We also documented if men underwent ADT by hormonal or surgical castration for prostate cancer. Participants also rated their overall health status compared to men their own age.

Smoking was defined as having smoked at least 100 cigarettes in their lifetime. Alcohol use was defined as having consumed 4 or more drinks per week in the past 12 months. Physical activity was assessed by whether participants walked for exercise, to work, the store or church in the past 7 days. We also used hours of television watching per week as a surrogate of physical inactivity. Dietary calcium intake was assessed by frequency of selected food items including fish, bone chewing, green leafy vegetables, beans, milk, cheese, and cheese dishes that contain high dietary calcium and are frequently consumed in the local diet.

5.3.4 Statistical Analysis

Measures of cortical bone were expressed as an annualized percentage rate of change from baseline (%/yr). Analysis of variance (ANOVA) was used to compare unadjusted annualized percentage rate of change in cortical bone across 5-yr age groups: 40 to 49, 50 to 54, 55 to 59, 60 to 64, 65 to 70, 70 and over. We evaluated the linear association of each predictor with measures of cortical bone at the radius and tibia separately using linear regression analysis with adjustment for age. The relationships between potential correlates and bone measures were expressed as a one

unit increase for categorical variables or approximately a one standard deviation (SD) increase for continuous variables, along with 95% confidence intervals, unless otherwise noted. To identify the independent correlates, separate multiple linear regression models were performed using a backwards elimination procedure for bone strength and geometry measures at the radius and tibia. Potential correlates with less than 5 observations were not considered for multiple linear regression models. A P-value of 0.10 in the univariate model was required to allow a variable to enter the multiple regression models. A P-value <0.05 was required for a variable to remain in the multiple regression model. Age was forced into the multiple regression models. To avoid invalid individual parameter estimations due to multi-collinearity, we entered only one variable from the same domain (e.g., “weight”, “waist” and “BMI”) into the multiple linear regression model based on the overall strength of the associations with all the skeletal outcomes. Independent correlates identified through the stepwise procedures were evaluated further for potential multi-collinearity using the variance inflation factor method. All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.4; SAS Institute, Cary, NC).

5.4 Results

The mean age of men included in our analytical sample was 56.8 ± 9.1 years (Table 1). Men experienced statistically significant changes in bone structure and geometry over the average 6.2 ± 0.5 years of follow-up (range 4.9 to 8.7 years) (Table 2). There was a significant linear trend for greater thinning of the cortices with advancing age across 5-year age groups at both skeletal sites (Figures 8 and 9). Bone strength estimated by SSI decreased 5% per decade in the tibia as compared to 0.8% per decade at the radius (Table 2; p-value < 0.001 at both sites). Periosteal

circumference increased 2% per decade in the radius and tibia, whereas endosteal circumference increased 9 – 10% per decade in both limbs (Table 2; p-value for all < 0.001). Cortical thickness decreased approximately 4% per decade at the radius and 5% per decade at the tibia. At the radius, the rate of periosteal expansion decreased resulting in cortical thinning across the 5-yr age groups (Figure 8; p-value for linear trend = 0.003). Similarly, at the tibia the rate of cortical thinning was significant for linear trend across the 5-yr age groups (Figure 9; p-value for linear trend = 0.001).

Correlates of change in bone strength and bone geometry in univariate analysis

At the radius, higher baseline body weight and higher baseline grip strength were associated with a slower rate of endosteal expansion and slower rate of cortical thinning (Table 3). A history of any type of cancer or prostate cancer, hypertension and smoking were also associated with greater cortical thinning. ADT use was associated with decreased cortical thickness and increased endosteal circumference. Use of insulin was associated with increased SSI and the use of non-steroidal anti-inflammatory drugs (NSAIDs) was associated with increased periosteal circumference.

At the tibia, higher baseline body weight was associated with a greater decline in SSI. Higher baseline body weight was related to less cortical thinning (Table 4). Smoking decreased rates of change in periosteal and endosteal circumferences. ADT was associated with cortical thinning. Among anti-hypertensive drugs: non-thiazide and beta-blocking agents increased and decreased, respectively, changes in periosteal and endosteal circumferences.

Independent correlates in Multivariable Models

At the radius, use of calcium channel blockers was associated with a greater loss of SSI, whereas use of insulin was associated with a gain in SSI (Table 5). Grip strength and diabetes were

associated with increased, and ADT with decreased, cortical thickness. Smoking and ADT were associated with increased endosteal circumference.

At the tibia, diabetes was associated with slower rate of decline in SSI (Table 6). Weight change, ADT, and use of warfarin were associated with decreased cortical thickness, whereas greater baseline height and use of thiazide diuretics were associated with increased cortical thickness. Warfarin use was associated with increased periosteal and endosteal circumferences, while having 3 or more chronic diseases and use of beta-blocking drugs was associated with decreased periosteal circumference. Greater baseline height, past smoking and use of thiazide diuretics were related to decreased endosteal circumference.

5.5 Discussion

As the world's older population increases, more men of African ancestry will develop osteoporosis and its associated fractures. (333) To better understand the natural history of skeletal aging in this population segment, we examined longitudinal changes in pQCT measures of cortical bone structure and strength, and their potential correlates in a large community-based sample of middle-aged and elderly African-ancestry men. To our knowledge, this is the first such study in men of African heritage and it also adds to the sparse longitudinal pQCT data among men in general. Endosteal expansion outpaced periosteal apposition in our cohort such that there was significant cortical thinning with advancing age. Moreover, the rate of cortical thinning accelerated with advancing age such that it was approximately two-fold greater at the radius among men aged 70 and older compared to those aged 40 to 49 years. This trend could be explained by the slowing of periosteal apposition amongst the oldest men whereas endosteal expansion appears to continue

unabated throughout life. These age patterns resulted in a net decrease in the strength-strain index, a measure of torsional bone strength. Additional studies are needed to determine the clinical significance of such changes, but these trends might contribute to the age-related increases in fracture incidence among African ancestry men. (223)

Our observed annual losses of 0.4% and 0.5% in cortical thickness at the radius and tibia respectively are consistent with the cross-sectional estimates of losses of 0.5%/year among white men aged 20 to 97 reported by Riggs *et al.* (77) Lauretani and colleagues longitudinally examined age-related changes in bone geometry at the mid-distal tibia in 345 Italian men participating in the InCHIANTI Study, aged 21 to 102, over a 6-year follow up period. Overall, total bone area, indicative of periosteal apposition, increased at a rate of 1.2%/decade, cortical bone area, indicative of cortical thinning, increased at a rate of 0.6%/decade and medullary area, indicative of endosteal resorption increased by 3.7%/decade. (105,106) Thus, this longitudinal study among Italian men showed that endosteal expansion exceeds periosteal expansion with advancing age resulting in a net loss of cortical bone.

We observed that the correlates of bone structural changes differed by anatomic site. Body weight is among the strongest correlates of DXA measures of BMD in cross-sectional studies of men. (283–288) Longitudinal studies have also demonstrated a strong and independent association of age-related weight loss with BMD loss in men including men of African ancestry. (300,342–345) However, the contribution of anthropometric measures to bone geometry and strength changes with aging in Caucasian or African ancestry men has not been well defined. In our study, higher body weight, BMI, and waist circumference were all associated with less cortical thinning in both limbs, which is not surprising given the strong correlation between body weight and BMD. (285,286,346)

We examined many medical history variables in the current analysis but few were related to changes in cortical bone geometry. We may have had limited power to detect true associations between some medical conditions with a low prevalence and changes in bone geometry. Diabetes remained a statistically significant correlate in age-adjusted and multivariable models. We found that diabetes prevalence was associated with a slower rate of decline in SSI at the tibia and a decreased rate of cortical thinning at the radius. Type 2 diabetics have increased fracture risk, (115,116), despite higher aBMD. (117–122) This paradoxical relationship is only partially explained by the increased propensity to falls. (123,124) Evidence from animal studies implicate diabetes-related deterioration in the material properties (128,347–349) and altered dynamics of bone (350–352) that predispose to fragility without affecting aBMD. Recent studies have shown enhanced trabecular BMD but compromised cortical bone strength and unfavorable cortical bone structure among type 2 diabetics. (120,353) These findings are also consistent with the associations between diabetes and cortical vBMD in our previous study. (354)

We observed that cigarette smoking was associated with slower endosteal resorption and periosteal apposition at the tibia. However, at the radius smoking was associated with an increased rate of endosteal resorption. These findings are consistent with the associations between smoking and cortical BMD in our previous study. (354) Lorentzon *et al.* found that smoking was associated with decreased cortical thickness in young men. (355) Furthermore, smoking has been associated with decreased endosteal and periosteal circumferences at the radius in Japanese men. (356) Most studies have found cigarette smoking to be associated with decreased aBMD. (285,300,342,343,355) However, the association with vBMD has been less consistent. (354,355,357)

We also examined several medications in relation to skeletal changes in the current study. We found that use of beta-blocking drugs, thiazides, NSAIDs and warfarin were significantly associated with skeletal changes in age-adjusted and multivariable models. Propranolol, a non-selective beta-blocker, has been shown to increase the rate of bone formation and the number of osteoblasts in rat models. (358) Furthermore, use of beta-blocking agents has been associated with a reduced risk of fracture. (359) However, we found that the use of beta-blocking agents was associated with a decreased rate of periosteal apposition at the tibia in age-adjusted models. Use of thiazide diuretics was associated with a lower rate of cortical thinning and endosteal resorption. Thiazides are known to be positively associated with aBMD and vBMD. (360,361) Considering the high prevalence of hypertension, particularly untreated hypertension, in African ancestry populations (362) these findings are important from a public health perspective. The use of NSAIDs was associated with an increased rate of periosteal apposition at the radius. NSAIDs suppress prostaglandin synthesis, which may influence bone formation directly. (363) Further, we found the use of warfarin, a Vitamin-K antagonist, to be associated with an increased rate of endosteal resorption and periosteal apposition at the tibia. Warfarin use has been associated with lower aBMD and increased fracture risk. (364,365) This relationship has been attributed to the abundance of osteocalcin in bone, a Vitamin-K –dependent protein responsible for the correct orientation of hydroxyapatite crystals. (366)

Our study findings may be limited by potential recall bias from questionnaire assessments; volunteer bias arising from our participants, who might differ from the general population; exclusive focus on the appendicular skeleton; and small numbers of men in the youngest and oldest age groups. Nonetheless, our study also has several notable strengths including a relatively large sample size, longitudinal study design, pQCT-derived measures of bone strength and bone

geometry, and a wealth of data on potential covariates. Moreover, the present study addresses a major gap in the literature on the patterns and correlates of bone structural and geometric changes with aging, particularly among African ancestry men who have been under-represented in past studies.

In conclusion, we found that the greatest age-related changes in appendicular skeletal structure among African ancestry men were in endosteal circumference reflecting endosteal resorption. The rate of change in endosteal circumference was similar in magnitude for both limbs and continued unabated with advancing age. On the other hand, periosteal apposition failed to compensate for endosteal resorption with advancing age, leading to a net loss of bone and cortical thinning. Torsional bone strength, as reflected in SSI, decreased at a more rapid rate in the tibia as compared to the radius. Correlates of bone geometry and strength differed by the measure and skeletal site examined. Additional longitudinal studies are needed to better define the biological mechanisms for age changes in bone geometry and strength at different skeletal sites, including the axial skeleton, in African ancestry populations. A better understanding of these factors could lead to new interventions to preserve bone strength with aging.

5.6 Tables and Figures

Table 1 Characteristics of African ancestry Men at Baseline (N=1,576)

Characteristics	Mean \pm SD or frequency (%)
Age (yr)	56.8 (9.1)
Anthropometry	
Body Weight (kg)	84.97 (14.81)
Height (cm)	175.5 (6.9)
BMI (kg/m ²)	27.5 (4.4)
Waist circumference (cm)	93.5 (11.01)

Table 1 continued

Grip Strength (kg)	44.4 (9.6)
Weight change (%)	-0.04 (6.93)
Waist circumference change (%)	5.4 (6.41)
Medical History (% yes; N)	
Hyperthyroid	0.8 (12)
Stroke	1.3 (21)
Heart attack	0.7 (11)
Congestive heart failure	1.2 (18)
CVD	3.3 (52)
Asthma	2.7 (42)
Lung disease	3.8 (59)
Anemia	0.9 (14)
Hepatitis	0.4 (6)
Any cancer	6.8 (106)
Prostate cancer	6.3 (99)
Kidney stone	1.9 (29)
Arthritis	11.6 (182)
Rheumatoid arthritis	1.3 (20)
Osteoarthritis	2.3 (36)
Fracture history	21.2 (327)
Hypertension	22.5 (355)
Diabetes	17.1 (261)
Back pain	42.9 (670)
Chronic diseases (3 or more)	4.5 (70)
Self-rated health (excellent/good)	94.3 (1467)
Lifestyle factors	
Walk in the past 7 days (yes)	62.0 (970)
Alcohol consumption (> 4 drinks/week)	10.3 (162)
Watching TV (>=14 hrs/week)	38.4 (599)
Smoking status	
Never	68.1 (1067)
Past	89.5 (1402)
Current	10.5 (164)
ADT	2.16 (34)
Medication use (% yes; N)	
NSAID	2.2 (35)
Thiazide diuretics	8.4 (132)
Non-thiazide diuretics	1.6 (25)
HMG CoA reductase inhibitors	1.1 (18)
Nitrates	0.4 (6)
Beta-blocking drugs	3.4 (53)

Table 1 continued

Diabetes medication	9.8 (154)
Anti-hypertension medication	19.3 (304)
Calcium channel blockers	5.5 (87)
Angiotensin-converting enzyme inhibitors	10.5 (165)
Angiotensin II receptor blockers	0.6 (10)
Proton pump inhibitors	0.6 (10)
Oral anticoagulant (not including anti-platelet)	0.3 (5)
Oral anticoagulant (including anti-platelet)	5.1 (81)
Warfarin (Vitamin K antagonists)	5.5 (86)

BMI-Body Mass Index; CVD- Cardiovascular disease; ADT-Androgen Deprivation Therapy; ACE - Angiotensin Converting Enzyme; NSAID-Nonsteroidal Anti-inflammatory Drugs.

Table 2 Rates of Change (%/yr) in Cortical Bone Structure and Strength over 6.2 years

Variable	Mean	Std Dev	P-Value
Radius			
Cortical Thickness	-0.43	0.95	<.0001
Periosteal Circumference	0.20	0.35	<.0001
Endosteal Circumference	1.09	2.11	<.0001
SSI	-0.08	0.95	<.0001
Tibia			
Cortical Thickness	-0.54	0.65	<.0001
Periosteal Circumference	0.23	0.25	<.0001
Endosteal Circumference	0.92	1.02	<.0001
SSI	-0.55	1.07	<.0001

SSI indicates strength-strain index

Table 3 Age-adjusted correlates of cortical bone structure and strength changes at the radius over 6.2 years (Beta coefficient and 95% confidence intervals)

Correlates	Unit	Polar SSI	Periosteal Circumference	Endosteal Circumference	Cortical Thickness
Age, unadjusted (yr)	9.14		-0.002 (-0.004, -0.001)		-0.01 (-0.02, 0.004)
Body Weight (kg)	14.81			-0.15 (-0.25, -0.04)	0.07 (0.02, 0.11)
Height (cm)	6.89			-0.15 (-0.26, -0.04)	
Grip Strength (kg)	9.57			-0.16 (-0.28, -0.03)	0.07 (0.02, 0.13)
Any cancer	Yes				-0.21 (-0.4, -0.02)
Prostate cancer	Yes				-0.22 (-0.41, -0.02)
Arthritis	Yes		0.05 (-0.01, 0.10)		
Hypertension	Yes				-0.09 (-0.19, 0.01)
Back Pain	Yes		0.03 (-0.01, 0.07)	0.19 (-0.02, 0.04)	-0.09 (-0.19, -0.003)
Diabetes	Yes	0.11 (-0.02, 0.24)			0.13 (0.0002, 0.25)
3 or more chronic diseases	Yes			0.5 (-0.01, 1.01)	-0.26 (-0.49, -0.03)
Ever smoked	Yes			0.21 (-0.1, 0.43)	-0.09 (-0.2, .01)
Currently smoke	Yes		-0.05 (-0.11, 0.01)		
ADT	Yes	-0.33 (-0.65, -0.001)		1.10 (0.37, 1.82)	-1.06 (-1.39, -0.74)
Oral hypoglycemics	Yes		-0.06 (-0.12, 0.001)		
Insulin	Yes	0.77 (0.06, 1.47)			
Calcium channel blockers	Yes	-0.27 (-0.47, -0.06)			
ACE Inhibitors	Yes				-0.14 (-0.3, 0.02)
NSAID	Yes		0.14 (0.02, 0.26)		
Warfarin (Vitamin K antagonists)	Yes	-0.72 (-1.55, 0.11)			

P-value <= 0.05 in bold. SSI indicates strength strain index

ADT - Androgen Deprivation Therapy; ACE - Angiotensin Converting Enzyme; NSAID - Nonsteroidal Anti-inflammatory Drugs

Table 4 Age-adjusted correlates of cortical bone structure and strength changes at the tibia over 6.2 years (Beta coefficient and 95% confidence intervals)

Correlates	Unit	Polar SSI	Periosteal Circumference	Endosteal Circumference	Cortical Thickness
Age, unadjusted (yr)	9.14				-0.005 (-0.01, -0.001)
Body Weight (kg)	14.81	-0.1 (-0.16, -0.04)			0.04 (0.005, 0.07)
Height (cm)	6.89		-0.01 (-0.03, 0.00)	-0.11 (-0.16, -0.06)	0.05 (0.01, 0.08)
Weight change (%)	6.41	-0.06 (-0.11, -0.002)			-0.05 (-0.08, -0.01)
Congestive heart failure	Yes		-0.10 (-0.22, 0.02)		
Osteoarthritis	Yes	0.32 (-0.04, 0.68)			
Diabetes	Yes	0.15 (0.01, 0.3)			
3 or more chronic diseases	Yes		-0.09 (-0.15, -0.03)		
Ever smoked	Yes		-0.04 (-0.07, -0.02)	-0.15 (-0.26, -0.04)	
Currently smoke	Yes		-0.04 (-0.08, 0.00)		
ADT	Yes				-0.19 (-0.42 – 0.04)
Thiazide diuretics	Yes			-0.21 (-0.4, -0.02)	0.15 (0.03, 0.27)
Non-thiazide diuretics	Yes		0.10 (-0.003, 0.21)	0.51 (0.07, 0.95)	
Beta-blocking agents	Yes		-0.09 (-0.17, -0.03)	-0.35 (-0.64, -0.05)	0.18 (-0.003, 0.37)
Warfarin (Vitamin K antagonists)	Yes		0.36 (0.12, 0.61)	2.10 (1.10, 3.09)	-0.68 (-1.32, -0.05)
NSAID	Yes		-0.09 (-0.18, 0.00)		

P-value <= 0.05 in bold. SSI indicates strength strain index

ADT – Androgen Deprivation Therapy; NSAID - Nonsteroidal Anti-inflammatory drugs

Table 5 Independent correlates of cortical bone structure and strength changes at the radius over 6.2 years (Beta coefficient and 95% confidence intervals)

Correlates	Unit	Polar SSI	Periosteal circumference	Endosteal circumference	Cortical Thickness
Age (yr)*	9.14	0.02 (-0.03, 0.07)	-0.03 (-0.04, -0.01)	-0.07 (-0.18, 0.04)	-0.03 (-0.08, 0.03)
Weight	14.81				0.05 (0.004, 0.10)
Height	6.89			-0.17 (-0.27, -0.06)	
Grip Strength	9.57				0.06 (0.01, 0.12)
Hypertension	Yes				-0.14 (-0.24, -0.04)
Diabetes	Yes				0.15 (0.02, 0.27)
Ever Smoked	Yes			0.25 (0.03, 0.48)	
ADT	Yes			1.05 (0.32, 1.78)	-1.03 (-1.36, -0.70)
Calcium-channel blockers	Yes	-0.31 (-0.51, -0.09)			
Insulin	Yes	0.92 (0.22, 1.63)			
NSAID	Yes		0.14 (0.02, 0.26)		

* Age was forced into the models; SSI indicates strength strain index

ADT - Androgen Deprivation Therapy; NSAID - Nonsteroidal Anti-inflammatory drugs

Table 6 Independent correlates of cortical bone structure and strength changes at the tibia over 6.2 years (Beta coefficient and 95% confidence intervals)

Correlates	Unit	Polar SSI	Periosteal circumference	Endosteal circumference	Cortical Thickness
Age (yr)*	9.14	-0.07 (-0.12, -0.01)	-0.01 (-0.02, 0.01)	-0.04 (-0.09, 0.02)	-0.05 (-0.09, -0.02)
Weight	14.81	-0.12 (-0.17, -0.06)			
Height	6.89			-0.11 (-0.16, -0.06)	0.05 (0.01, 0.08)
Weight change	6.93				-0.05 (-0.08, -0.02)
Ever Smoked	Yes		-0.04 (-0.07, -0.02)	-0.13 (-0.24, -0.03)	
Diabetes	Yes	0.21 (0.06, 0.36)			
More than 3 chronic diseases	Yes		-0.09 (-0.15, -0.02)		
ADT	Yes				-0.31 (-0.54, -0.08)
Thiazides	Yes			-0.23 (-0.42, -0.04)	0.17 (0.05, 0.29)
Beta blocking drugs	Yes		-0.10 (-0.17, -0.03)		
NSAID	Yes		-0.09 (-0.18, -0.001)		
Warfarin	Yes		0.35 (0.11, 0.59)	2.02 (1.04, 3.01)	-0.68 (-1.31, -0.05)

* Age was forced into the models; SSI indicates strength strain index

ADT - Androgen Deprivation Therapy; NSAID - Nonsteroidal Anti-inflammatory drugs

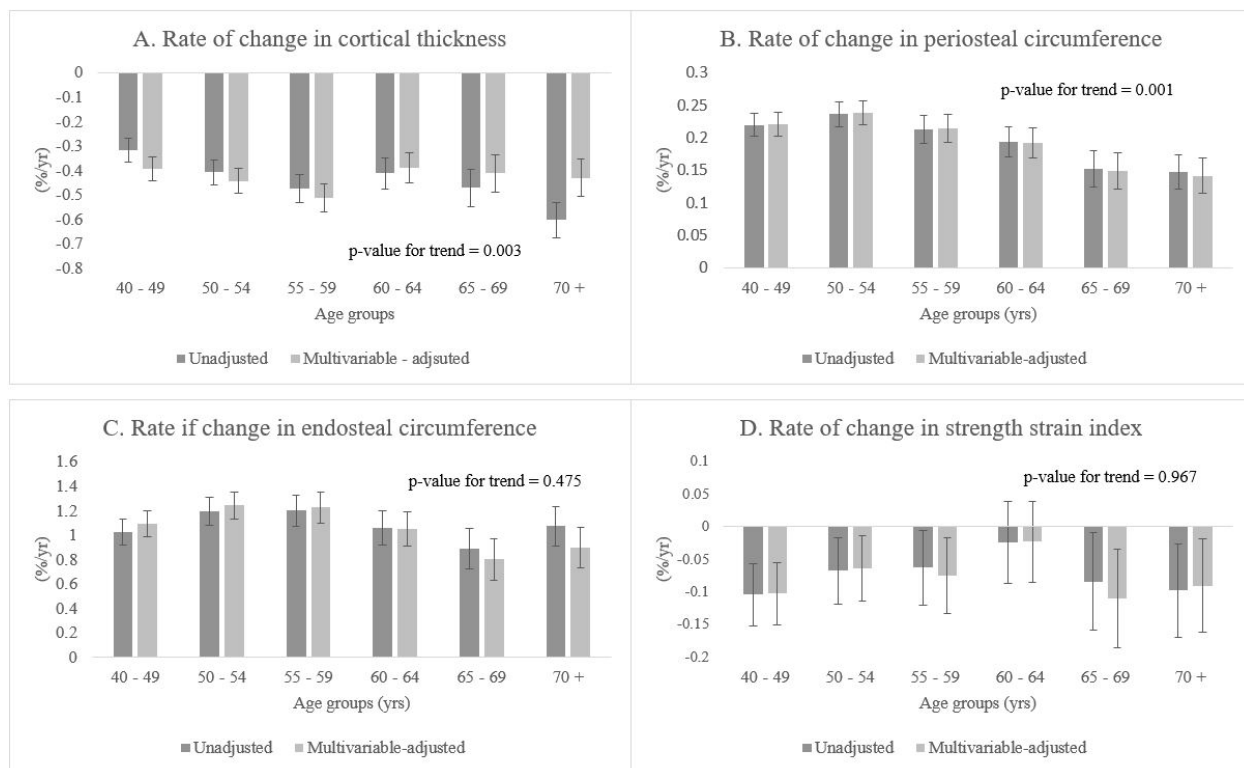


Figure 8 Rates of change in cortical bone structure and strength at the radius across 5-yr age groups
 Cortical thickness adjusted for age, weight, grip strength, history of hypertension, diabetes and use of ADT;
 Periosteal Circumference adjusted for age and use of NSAIDs; Endosteal Circumference adjusted for age, height, past smoking and ADT; Strength Strain Index was adjusted for age, use of calcium-channel blockers and insulin.

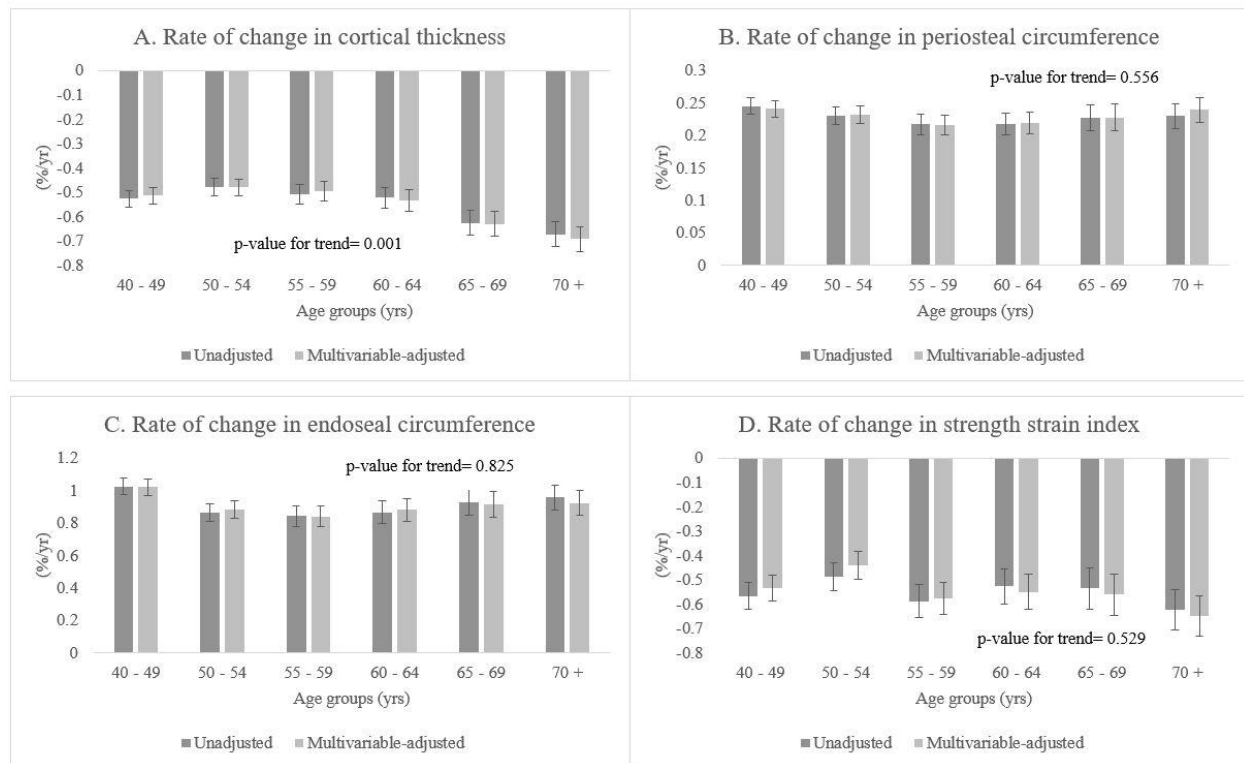


Figure 9 Rates of change in cortical bone structure and strength across 5-yr age groups at the tibia

Cortical thickness adjusted for age, height, weight change, use of thiazides, use of warfarin and ADT;

Periosteal circumference was adjusted for age, past smoking, having 3 or more chronic diseases, use of NSAIDs, use of beta-blocking drugs and warfarin; Endosteal circumference was adjusted for age, height, past smoking, use of thiazides and warfarin; Strength Strain Index was adjusted for age, weight and history of diabetes.

6.0 Cross-sectional and Longitudinal Associations between inflammation markers and cortical bone density, structure and strength in African Ancestry men

6.1 Abstract

Osteoporosis is a well-known consequence of inflammatory joint and bowel diseases. Some studies examining the link between fracture risk and low-grade inflammation have shown that inflammatory markers predicted fractures independent of bone mineral density (BMD). We tested the hypothesis that higher levels of biomarkers of inflammation compromise cortical bone structure. We assessed serum levels of Interleukin-6 (IL6) and high sensitivity C-reactive protein (hsCRP), and changes in cortical bone structure in 582 African ancestry men aged ≥ 40 years (59.08 ± 10.51 years) during an average follow-up of 6.2 years. To gain a better understanding of skeletal health in African ancestry men, we examined changes in cortical BMD, cortical thickness, periosteal and endosteal circumferences, and strength strain index (SSI), a measure of bone's torsional strength, using peripheral quantitative computed tomography (pQCT) at the radius and tibia. In cross-sectional analyses, we found negative associations between hsCRP and SSI and periosteal circumferences at the radius and tibia (all $p < 0.05$). HsCRP was not associated with rates of change in bone structure and strength. IL6 was negatively associated with cortical thickness when examined cross-sectionally. Contrary to our hypotheses, higher serum IL6 was significantly associated with slower rates of loss of cortical bone density and SSI at the radius and tibia. These associations remained after adjustment for anthropometric and lifestyle factors. We conclude that serum hsCRP levels are not related to changes in cortical bone structure in African ancestry men.

The pro and anti-inflammatory effects of IL6 could partially explain the inverse relationship found between serum IL6 and rates of change in cortical bone structure.

6.2 Introduction

Osteoporosis continues to be underdiagnosed and undertreated in men (7,8) even though one in eight men over the age of 50 will experience an osteoporosis-related fracture in their lifetime, (134) and compared to women men experienced higher morbidity and mortality after fractures. (135–137) As the world population ages and longevity improves, a better understanding of male osteoporosis is vital as osteoporosis-related fractures are preventable. Rodent studies and laboratory data suggest that IL6, IL1 and TNF α play important roles in bone remodeling and the pathogenesis of osteoporosis. (252,253) The epidemiologic evidence examining the link between low-grade inflammation and fracture risk comes from studies mostly focused on Caucasians usually using measures of CRP or hsCRP. (263,264,266–268,276) The data linking aBMD alterations as the mechanism linking fracture risk and biomarkers of inflammation have been inconsistent. Some have found an independent relationship (263–265,267); others that have reported an inverse relationship (273,307); and yet others have found no relationship. (310–312) One potential explanation for this discrepancy is the limitation of DXA in capturing aspects of bone quality. To our knowledge the relationship between biomarkers of inflammation and changes in cortical bone structure has not yet been examined in men of African ancestry, who have been under-represented in studies of bone loss.

Thus, we hypothesized that higher levels of inflammation markers, denoted by elevated levels of IL6 and hsCRP, will be associated with greater rate of loss in cortical BMD and SSI, and increased cortical thinning due to increased endosteal expansion and reduced periosteal expansion.

6.3 Methods

6.3.1 Study population

Between 1997 and 2003, 3,170 men aged 40 and older were recruited for population-based prostate cancer screening for the first time on the island of Tobago, Trinidad & Tobago. (31) To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment for the initial screening was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island parishes. The recruited cohort was 97% African, 2% East Indian, <1% white, and <1% "other" as defined by participant-report of paternal and maternal grandparents' ethnicity. The low non-African admixture in this population (6%) has been confirmed using ancestry informative genetic markers. (32)

Between 2004 and 2007, men in the original cohort were invited to complete a peripheral QCT (pQCT) scan as part of the Tobago Bone Health Study. An additional 451 new participants were also recruited using similar methods and enrollment criteria as the initial screening study. A total of 2174 men underwent pQCT scans of the radius and tibia at this exam. (33) Between 2010 and 2013, we invited these men to return for repeat pQCT scans. Both the baseline and follow-up

visits followed the same procedures for questionnaire interviews, biospecimen collection, pQCT and DXA scans. (33) A total of 1576 African ancestry men completed the follow-up pQCT exam (80% of survivors). For the current analysis, we selected a sample of 582 men for serum measures of inflammation markers. We excluded men who were not of African ancestry. The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study and all participants provided written informed consent before data collection.

6.3.2 Peripheral QCT

QCT scans were performed at the non-dominant forearm and left tibia using the Stratec XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany). Technicians followed a standardized protocol for patient positioning and scanning at both the baseline and follow-up visits. A scout view was initially obtained prior to the baseline pQCT scan to define an anatomic reference line for the relative location of the subsequent scans at the radius and tibia. Tibia length was measured from the medial malleolus to the medial condyle of the tibia, and forearm length was measured from the olecranon to the ulna styloid process. Scans were taken at 33% of the total length of forearm and tibia. Measurements at the 33% sites represent predominantly cortical bone. A single axial slice of 2.5 mm thickness with a voxel size of 0.5 mm and a speed of 20 mm/s was taken at all locations. Image processing was performed by a single investigator using the Stratec software package (Version 5.5E). All 33% radius and tibia scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm³; peel mode 2; Cortmode 1) to determine bone geometric properties. SSI was determined with Cortmode 1 (T = 280mg/cm³). Cortical thickness (mm), periosteal circumference (mm),

endosteal circumference (mm) and polar SSI (SSI, mm³) were measured at both radius and tibia. Coefficients of variation were determined for pQCT scans by replicating measurements on 15 subjects with $CV \leq 2.1\%$ for all measures. Daily phantom scans were analyzed to ensure long-term scanner stability. It is not possible to estimate material properties like elastic modulus from QCT measurements. However, vBMD measured by QCT has an approximately linear relationship with elastic modulus. Based on this relationship, Schiessl *et al.* (34) developed the SSI, which is the integrated product of section modulus and vBMD normalized to the maximal physiological cortical vBMD of human bones. Section modulus (mm³) was first calculated as $(a \times d^2)/d_{max}$, where a is the cross-sectional area of a voxel (mm²), d is the distance of the voxel from the center of gravity (mm), and d_{max} is the maximum distance or eccentricity of any voxel to the center of gravity (mm). The ratio of cortical vBMD and normal physiological vBMD (1200 mg/mm³) provides an estimate of the modulus of elasticity.

6.3.3 Biomarkers of Inflammation

HsCRP was measured using reagents obtained from Beckman-Coulter (Brea, CA) and analyzed on an AU400 from Olympus America, Inc. (Melville, NY). In this procedure the CRP in the sample reacts with goat anti-CRP-antibodies coated on latex particles. The increase in absorbance is measured turbidimetrically. Blanks, controls and standards (0.5 to 20 mg/L) are run simultaneously with all samples. For this study, the intra- and inter-assay coefficients of variation were 5.5% and 3.0%, respectively.

IL6 was measured, in duplicate, using a commercial high sensitivity ELISA kit (R&D Systems, Minneapolis, MN). In brief, samples were incubated at room temperature (RT) for 3 hours in microplate wells coated with murine monoclonal antibody against IL6. The plates were

washed (x4), 200 µl of conjugate (alkaline phosphatase/polyclonal antibody against IL6) added and the samples further incubated for 2 hours at RT. The plates were again washed (x4) and 50 µl of substrate (NADPH) then added. The samples were incubated for 60 minutes at RT, 50 µl of amplifier (alcohol dehydrogenase/diaphorase) added and the plates incubated for 30 minutes at RT. The reaction was stopped with sulfuric acid (50 µl of 2N) and the absorbance read at 490 nm with correction at 650 nm. Standards (0.15 to 10 pg/mL), controls and a pooled laboratory control were run with each assay. For this study, the intra and inter-assay coefficients of variation were 9.1% and 10.2%, respectively.

6.3.4 Anthropometric measurements

Body weight was measured in kilograms with participants wearing light clothing and without shoes using a calibrated balance beam scale. Height was measured in centimeters without participants wearing shoes using a wall-mounted height board. Two height measurements were made and the average used in analysis.

6.3.4.1 Other measurements

Trained interviewers and nurses administered questionnaires to participants. We focused on potential correlates of bone strength and bone geometry based on the literature and from our previous findings of correlates for BMD in this cohort. (29,30) We collected information pertaining to demographic characteristics, medical history, medication use, personal and family fracture history, physical activity, and lifestyle habits. Ethnicity was self-reported and participants provided detailed information on the ethnic origin of their parents and grandparents.

Participants were asked if they had been diagnosed with cardiovascular diseases, diabetes, respiratory diseases, hypertension, cancer, and fractures. Family history of fracture was also ascertained. Personal history of diabetes was defined as having a history of using diabetes medications or a fasting glucose level of 126mg/dL and above. Medication use was coded based on the Anatomical Therapeutic and Chemical and Defined Daily Dose system. (341)

Smoking was defined as having smoked at least 100 cigarettes in their lifetime. Alcohol use was defined as having regularly consumed 4 or more drinks per week in the past 12 months. We used hours of television watching per week as a surrogate of physical inactivity.

6.3.5 Statistical analysis

Longitudinal measures of cortical bone were expressed as an annualized percentage rate of change from baseline (%/yr). We evaluated IL6 and hsCRP for normality and performed log transformations. We reported the beta coefficient of the relationships between markers of inflammation and bone measures, which were expressed per one SD increase in log-transformed values of inflammation markers, along with 95% confidence intervals, unless otherwise noted. We also assessed the association between markers of inflammation and potential covariates using multiple linear regression, we fit age-adjusted, age, weight and height-adjusted and fully-adjusted models incorporating lifestyle factors. Twenty-nine men having hsCRP values higher than 7.5mg/L, suggesting active inflammation, were excluded from the analysis yielding an analytical cohort of 553 African ancestry men. We also examined hsCRP and IL6 categorized by clinical cut points and quantiles as appropriate. We carried out sensitivity analyses by excluding men with and without diabetes and men aged 60 and older. All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.4; SAS Institute, Cary, NC).

6.4 Results

The 553 men in the subsample were aged 40 and over with the average age being 59 ± 10.5 years. Baseline characteristics of the men in the sample are shown in Table 7. The serum level of hsCRP and IL6 were 1.55 and 2.51 mg/L respectively. Log-transformed hsCRP and IL6 were significantly associated with age, weight, and weight change (data not shown).

Periosteal circumference and Endosteal circumference increased significantly from baseline and changes were of similar magnitude at the radius and tibia. Overall, there were significant reductions in cortical thickness and SSI at the radius and tibia (Chapter 5.4).

Cross-sectional associations between baseline hsCRP and IL6 and cortical bone density, structure, and strength

At the radius and the tibia, higher baseline hsCRP was negatively associated with periosteal circumference and SSI (p-value < 0.05). These relationships remained significant after adjusting for baseline anthropometric and lifestyle factors like current smoking, physical inactivity, and alcohol consumption. A significant negative association was also seen with endosteal circumference, but only at the radius (Table 8).

We found a negative association between higher baseline IL6 and cortical thickness at the tibia that persisted even after adjustment for weight, height and lifestyle factors. We found no other cross-sectional association between IL6 and measures of cortical BMD, structure and strength at either skeletal site. (Table 8).

Association between baseline hsCRP and IL6 and rate of change in cortical bone density, structure and strength

No association was seen between baseline hsCRP and annual rates of change in cortical BMD, structure or strength at either the radial or tibial sites. Conversely, higher baseline IL6 was

associated with lower rates of change in cortical BMD and SSI at the radius and tibia even in fully-adjusted models (Table 9).

6.5 Discussion

To our knowledge the relationship between biomarkers of inflammation and cortical bone geometry, particularly in African ancestry men, has not been examined. Moreover, studies of inflammation markers in relation to QCT-measured bone attributes, particularly in men have been rare. (275) We found that higher serum levels of hsCRP and IL6 were associated with lower PeriC and SSI in cross-sectional analyses consistent with our hypotheses; however, baseline hsCRP showed no association with rates of change in cortical bone measure, while baseline IL6 showed an unexpected positive association with cortical BMD and SSI.

While higher serum hsCRP was negatively associated with SSI in cross-sectional analyses, no such relationship was observed with changes in SSI. In contrast, higher serum IL6 was associated with a slower rate of loss in SSI and cortical BMD at both skeletal sites even after multivariable adjustment. It is noteworthy that in normal physiological states, IL6 can have anti-inflammatory functions via stimulating IL1- receptor antagonist, an anti-inflammatory mediator. (367) Therefore, it is possible serum IL6 levels could represent net pro- or anti-inflammatory activities.

The overlapping biological pathways between bone and inflammation lend support to the idea that osteoporosis results partly from an inflammatory process. (260–262) Two physiological pathways potentially favoring osteoclastogenesis explain this relationship: pro-inflammatory cytokines increase the expression of RANKL, decrease OPG, and increase M-CSF in stromal cells

(254); and estrogen deficiency increases the rate of bone resorption by increasing osteoclast numbers. (255,256) It is noteworthy that other well-known risk factors for fractures like smoking and low physical activity are mediated through inflammation. (257–259)

As inflammation biomarkers predicted fracture risk independent of aBMD in some studies, impairments in bone microarchitecture were hypothesized as the underlying mechanism. Rolland and colleagues (275) tested this hypothesis by evaluating the relationship between hsCRP and HR-pQCT-measured bone microarchitecture in 1149 men aged 19 – 87 years from the STRAMBO cohort. In men over the age of 72, they found that higher hsCRP was associated with unfavorable trabecular microarchitecture. (275) However, they did not report any cross-sectional association between inflammation markers and cortical microarchitecture in older men. (275) While our study sample was limited by small numbers of men in the older age groups as well as lack of information on cortical and trabecular microarchitecture, we found no cross-sectional association between hsCRP or IL6 and trabecular BMD (data not shown). Although, Ishii and colleagues did not assess bone microarchitecture, they reported hsCRP was negatively associated with DXA-measured bone strength and not aBMD in a longitudinal study of women aged 75 and over with a 10-year follow-up period, attributing these findings to inflammation-mediated disruption of the balance between bone strength and mechanical load. (266) Our cross-sectional analyses revealed similar associations.

Eriksson and colleagues (274) examined the relationship between hsCRP and incident fractures in 69 to 81 - yr old men from the MrOS study in Sweden. When comparing the highest tertile of log transformed hsCRP with the lower two combined, the adjusted risk for all fractures was 48% higher (HR 1.48, 95% CI 1.20 – 1.82). The risk was 61% higher for vertebral fractures (HR 1.61, 95% CI, 1.12 – 2.29). These relationships remained after adjustment for aBMD,

suggesting mechanisms independent of BMD. After excluding fractures within the first three years of follow-up, the relationships between hsCRP and incident fracture grew stronger for all fractures (HR 1.61, 95% CI 1.18 – 2.20), as well as, for vertebral fractures only (HR 1.89, 95% CI 1.19 – 3.02). This augmentation of the association is suggestive of the effect of persistent low-grade inflammation. (274) Our analyses are limited by a single baseline measure of inflammation markers.

Some studies have assessed more than one inflammation marker. (267,268,273,278) Sponholtz and colleagues (278) used data from the Framingham Offspring Study to examine the cross-sectional relationship between inflammation and BMD in 1293 middle-aged to older men. They found no relationship between IL6 or TNF α and BMD after adjustment for height, weight, smoking and physical activity, leading them to suggest that the measurement of inflammatory markers may be of little utility in identifying individuals at higher risk of fracture. Serum levels of cytokine biomarkers may not accurately reflect tissue-level activities of the same, (269) and elevations of soluble receptor levels may represent more severe inflammation (269,270). By assessing serum levels of SRs of IL6 and TNF- α (TNF SR1 and TNF SR2) Barbour and colleagues (268) addressed limitations posed by the transient nature of elevated IL6 and TNF α levels and reported that having elevated levels of all three cytokine receptors was associated with 1 SD decline in BMD (271) in the WHI cohort.

Only one study has examined the rate of change in aBMD in association with serum levels of inflammation markers (273); it found baseline IL6, hsCRP and TNF α significantly predicted 3-year loss in DXA-measured total body aBMD after adjustment for age, sex, anthropometry, and smoking in 168 older adults aged 50 – 79 from the Tasmania Older Adult Study. Furthermore, 3-year change in IL6 was a significant predictor of greater decline in aBMD at the hip and spine;

similarly, TNF α was a significant predictor of changes in aBMD at the spine. Lastly, in models including all the markers of inflammation, IL6 emerged as an independent predictor of BMD change. (273)

A major limitation of our study is the availability of one single measurement of hsCRP and IL6; the utility of the latter in particular has been called into question. (313) Only one study has assessed the relationship between changes in levels of inflammation markers and annual losses of BMD and found higher IL6 to be an independent predictor of bone loss. (273) Our findings pertaining to IL6 could potentially be explained by: the limitation of one single measure of inflammatory markers; lack of information on the duration of low-grade inflammation; and limited understanding of the biological role of IL6. Furthermore, our study findings may be limited by potential recall bias from questionnaire assessments of lifestyle factors; volunteer bias arising from our participants, who might differ from the general population; and exclusive focus on the appendicular skeleton. Nonetheless, our study also has several notable strengths including a longitudinal study design, pQCT-derived measures of bone strength and bone geometry, and a wealth of data on potential covariates. Moreover, the present study addresses a major gap in the literature pertaining to the relationship between biomarkers of inflammation and bone structural and geometric changes with aging, particularly among African ancestry men who have been under-represented in past studies.

In conclusion, we found that baseline hsCRP was not a predictor of changes of cortical bone structure. Serum levels of IL6 likely reflect the net of its pro and anti-inflammatory activities potentially explaining the inverse relationship observed with changes in cortical bone density and torsional bone strength. Additional studies are needed to better understand the physiological mechanisms underlying skeletal changes with aging in men.

6.6 Tables and Figures

Table 7 Baseline Characteristics of Tobago Bone Health Study subsample

Characteristic (n = 553) ¶	Mean (SD)
Age(yrs)	59.08 (10.51)
Height (cm)	174.58 (6.73)
Weight (kg)	83.18 (13.94)
Percentage weight change (%) *	-0.11 (7.35)
Ever Smoked (Y/N)	29.08 (171)
Alcohol consumption (4 or more alcoholic drinks/ week in last 12 months (Y/N)	8.84 (52)
Physical Inactivity (Watching TV for 14 hours or more/ week) (Y/N)	37.95 (222)
Diabetes (Y/N)	21.09 (124)
Degenerative arthritis (Y/N)	2.7 (16)
Having 3 or more chronic conditions (Y/N)	4.6 (27)
hsCRP (mg/L)	1.55 (1.42)
IL6 (pg/ml)	2.51 (2.10)
Baseline bone density, structure and strength at radius	
Cortical density (mg/cm ³)	1212.74 (24.57)
Cortical thickness (mm)	3.64 (0.37)
Periosteal circumference (mm)	43.51 (3.07)
Endosteal circumference (mm)	20.63 (3.73)
Strength Strain Index (mm ⁴)	421.52 (81.85)
Baseline cortical density, structure and strength at tibia	
Cortical density (mg/cm ³)	1177.36 (25.59)
Cortical thickness (mm)	5.61 (0.81)
Periosteal circumference (mm)	80.19 (5.50)
Endosteal circumference (mm)	44.97 (7.28)
Strength Strain Index (mm)	2543.01 (474.84)
Changes in cortical density, structure and strength at radius (%/yr)	
Cortical density	-0.21 (0.23)
Cortical thickness	-0.39 (0.88)
Periosteal circumference	0.19 (0.32)
Endosteal circumference	0.93 (1.82)
Strength Strain Index	-0.02 (0.92)
Changes in cortical density, structure and strength at tibia (%/yr)	
Cortical density	-0.22 (0.19)
Cortical thickness	-0.56 (0.48)
Periosteal circumference	0.25 (0.20)
Endosteal circumference	0.91 (0.70)
Strength Strain Index	-0.55 (1.04)

hsCRP - High sensitivity C-reactive protein; IL6 – Interleukin-6

Table 7 continued

* weight change at follow-up compared to baseline weight expressed as a percentage

‡ - 10 men have either serum hsCRP or IL6 measures not both;

Beta coefficient per SD increase in log transformed hsCRP

Excluded 29 men with hsCRP levels > 7.5 mg/ml indicating active inflammation

Table 8 Adjusted cross-sectional and longitudinal associations of serum hsCRP with cortical bone density, structure and strength at radius and tibia (n = 553)

Baseline cortical density, structure and strength at the radius	Multivariable-β (95% CI) *	Multivariable-β (95% CI) **
Density (mg/cm ³)	0.23 (-1.83 – 2.30)	0.30 (-1.75 – 2.34)
Thickness (mm)	0.01 (-0.03 – 0.04)	0.01 (-0.3 – 0.04)
Periosteal circumference (mm)	-0.32 (-0.57 – -0.07)	-0.31 (-0.56 – -0.06)
Endosteal circumference (mm)	-0.37 (-0.68 – -0.04)	-0.35 (-0.67 – -0.03)
Strength Strain Index (mm ⁴)	-7.67 (-14.43 – -0.91)	-7.75 (-14.51 – -0.99)
Changes in cortical density, structure and strength at the radius (%/yr)		
Density	0.02 (-0.003 – 0.04)	0.02 (-0.004 – 0.04)
Thickness	-0.01 (-0.09 – 0.07)	-0.01 (-0.09 – 0.07)
Periosteal circumference	0.01 (-0.02 – 0.03)	0.004 (-0.02 – 0.03)
Endosteal circumference	-0.001 (-0.17 – 0.17)	-0.002 (-0.17 – 0.17)
Strength Strain Index	0.03 (-0.05 – 0.11)	0.04 (-0.05 – 0.12)
Baseline cortical density, structure and strength at the tibia		
Density (mg/cm ³)	1.40 (-0.75 – 3.55)	1.57 (-0.55 – 3.68)
Thickness (mm)	-0.03 (-0.10 – 0.04)	-0.03 (-0.10 – 0.04)
Periosteal circumference (mm)	-0.74 (-1.17 – -0.033)	-0.76 (-1.18 – -0.034)
Endosteal circumference (mm)	-0.54 (-1.15 – 0.06)	-0.58 (-1.18 – 0.02)
Strength Strain Index (mm ⁴)	-64.84 (-101.55 – -28.13)	-64.82 (-101.64 – -28.01)
Changes in cortical density, structure and strength at the tibia (%/yr)		
Density	0.01 (-0.01 – 0.28)	0.01 (-0.01 – 0.28)
Thickness	0.03 (-0.01 – 0.08)	0.03 (-0.01 – 0.08)
Periosteal circumference	-0.01 (-0.02 – 0.01)	-0.01 (-0.02 – 0.01)
Endosteal circumference	-0.03 (-0.1 – 0.03)	-0.03 (-0.1 – 0.03)
Strength Strain Index	0.01 (-0.09 – 0.1)	0.0001 (-0.09 – 0.91)

*- Adjusted for age, height, weight and weight change

**-. Additionally, adjusted for lifestyle factors at baseline

p-value < 0.05 in bold

Table 9 Adjusted cross-sectional and longitudinal associations of serum IL6 with cortical bone density, structure and strength at radius and tibia (n = 553)

Baseline cortical density, structure and strength at the radius	Multivariable- β (95% CI) *	Multivariable- β (95% CI) **
Density (mg/cm ³)	1.35 (-0.61 – 3.32)	1.28 (-0.66 – 3.22)
Thickness (mm)	0.003 (-0.03 – 0.03)	0.004 (-0.03 – 0.04)
Periosteal circumference (mm)	-0.12 (-0.36 – 0.11)	-0.12 (-0.35 – 0.12)
Endosteal circumference (mm)	-0.15 (-0.44 – 0.15)	-0.15 (-0.45 – 0.15)
Strength Strain Index (mm ⁴)	-4.35 (-1.072 – 2.01)	-4.02 (-10.38 – 2.33)
Changes in cortical density, structure and strength at the radius (%/yr)		
Density	0.04 (0.02 – 0.06)	0.04 (0.02 – 0.06)
Thickness	-0.01 (-0.08 – 0.07)	-0.01 (-0.09 – 0.06)
Periosteal circumference	-0.001 (-0.03 – 0.03)	-0.001 (-0.03 – 0.03)
Endosteal circumference	-0.02 (-0.18 – 0.14)	-0.01 (-0.17 – 0.14)
Strength Strain Index	0.09 (0.01 – 0.16)	0.09 (0.12 – 0.17)
Baseline cortical density, structure and strength at the tibia		
Density (mg/cm ³)	0.78 (-1.28 – 2.84)	0.80 (-1.23 – 2.82)
Thickness (mm)	-0.08 (-0.15 – -0.02)	-0.08 (-0.15 – -0.01)
Periosteal circumference (mm)	-0.17 (-0.57 – 0.23)	-0.18 (-0.58 – 0.23)
Endosteal circumference (mm)	0.36 (-0.21 – 0.92)	0.32 (-0.25 – 0.89)
Strength Strain Index (mm ⁴)	-33.65 (-68.89 – 1.59)	-33.24 (-68.59 – 2.11)
Changes in cortical density, structure and strength at the tibia (%/yr)		
Density	0.04 (0.02 – 0.05)	0.03 (0.02 – 0.05)
Thickness	0.02 (-0.02 – 0.06)	0.02 (-0.02 – 0.06)
Periosteal circumference	0.002 (-0.02 – 0.02)	0.002 (-0.02 – 0.02)
Endosteal circumference	-0.04 (-0.1 – 0.02)	-0.04 (-0.1 – 0.02)
Strength Strain Index	0.13 (0.05 – 0.22)	0.13 (0.04 – 0.22)

*- Adjusted for age, height, weight and weight change for measures of change

**- Additionally, adjusted for lifestyle factors at baseline

p-value < 0.05 in bold

7.0 Relationship between calf adiposity and changes in cortical bone strength and geometry in African Ancestry men

7.1 Abstract

Recent evidence has shown that adipose tissue has distinct effects on skeletal health depending on its regional distribution. Few have examined the longitudinal relationship between ectopic depots of adipose tissue and bone loss. We evaluated the relationship between quantitative computed tomography-measured total adipose tissue (TAT) at the calf and changes in cortical BMD and bone structure (cortical thickness, periosteal circumference and endosteal circumference); and SSI at the tibia in 1576 African Ancestry men aged 40 and over, during an average follow-up of 6.2 years. We tested for associations between quartiles of TAT depots and changes in cortical bone measures using multiple linear regression. Higher TAT at the calf was associated with greater rates of loss in BMD and SSI (p-value for linear trend < 0.05 for both). The associations remained or were slightly attenuated after adjusting for age, height, weight loss from baseline, and lifestyle factors (p for trend < 0.05 for all). However, on additionally adjusting for baseline body weight, the association was no longer significant for SSI, but augmented for cortical BMD (p for trend < 0.0001). Further, reciprocal relationships were observed between percentage subcutaneous adipose tissue (%SAT) and percentage non-subcutaneous adipose tissue (% NSAT) at the calf with cortical BMD, showing decreased and increased rates of cortical BMD loss that remained significant after multivariable adjustment. Higher %SAT was associated with greater periosteal expansion, persisting in fully-adjusted models. Moreover, less fatty and more dense calf

muscle was associated with a slower rate of cortical BMD loss independent of total body weight. Additional research is needed to elucidate the biological pathways driving these associations.

7.2 Introduction

Osteoporosis and obesity are highly prevalent public health conditions attributed to the dysregulation of a common precursor cell. (368) In the past couple of decades, some paradigm shifts have occurred in public health and medical research: firstly, adipose tissue is no longer considered an inert depot of energy, but a dynamic endocrinologically active tissue (369); the potential for healthy aging has been increasingly recognized; osteoporosis in males is recognized as an important public health issue (145,370,371); global prevalence of obesity has risen and continues to rise at an alarming rate (372,373); and lastly, obesity and osteoporosis, two seemingly unrelated conditions, have been shown to share several characteristics. (368) Epidemiological studies have consistently reported higher body weight (283–288) and higher lean mass (289–292) to be positive determinants of BMD in cross-sectional studies of men. The positive association of higher body weight and its protective effect against fractures is well known, making body weight a determinant in the FRAX algorithm to compute fracture risk. (374) Recent evidence has challenged this paradigm by examining the individual effects of fat mass and lean mass on skeletal health. (220,301,302)

With aging, a process of redistribution of fat tissue occurs leading to infiltration of visceral organs (375,376) like the pancreas (377) and muscle (378); this infiltrated fat tissue is collectively called ectopic fat. Using QCT- based measures of fat and bone quality, Gilsanz and colleagues showed that SAT and VAT had positive and negative associations respectively with femoral

structure in young women. (303) These associations have been attributed to the unfavorable hormonal profile and higher inflammatory activity associated with visceral fat. (379,380) The biological mechanisms underlying these associations are complex operating in both directions. The positive effects of obesity occur through various modes including: mechanical loading, which leads to favorable biomechanical changes; increased peripheral generation of estrogen, which is inhibitory to bone resorption; and hyperinsulinemia, which is favorable for bone formation. (381) Conversely, the negative effects of obesity include being a pro-inflammatory state and potentially unfavorable biomechanical changes. (381)

Few studies have examined the relationship between fat and bone loss characterized in terms of bone structure and strength. (303–305) Yerges-Armstrong and colleagues have previously shown reciprocal relationships between SAT and NSAT at the calf with QCT-measured cortical and trabecular BMD in a cross-sectional analysis. (304) To further investigate the relationship between rates of cortical bone loss and calf TAT, we hypothesized that over a follow-up period of 6.2 years, African ancestry men aged 40 and over with higher calf TAT would have: greater rates of cortical density loss, higher rate of cortical thinning; greater rate of endosteal expansion; lower rate of periosteal expansion; and higher rate of loss of bone strength or SSI.

7.3 Methods

7.3.1 Study population

From 1997 to 2003, 3170 men aged 40 and over were recruited for a population-based prostate cancer screening in Tobago, Trinidad & Tobago. Recruitment was accomplished

predominantly by word of mouth, as well as flyers, public service announcements, posters and through healthcare workers at local hospitals and health centers (335). The recruited men represented approximately 60% of all age-eligible men on the island, who had to be ambulatory, non-institutionalized and not terminally ill to be eligible for participation. Based on participant self-report of paternal and maternal grandparents' ethnicity, the recruited cohort was 97% African, 2% East Indian, <1% white, and <1% "other". The low non-African admixture in this population (6%) has been confirmed using ancestry informative genetic markers. (336)

Men from the original cohort were invited to complete DXA and pQCT scans between 2004 and 2007 as part of the Tobago Bone Health Study. Additionally, 451 new participants were also recruited using similar methods and enrollment criteria as the initial screening study. A total of 2174 men underwent pQCT scans of the radius and tibia at this exam (340). Between 2010 and 2013, we invited these men to return for repeat pQCT scans. Both the baseline and follow-up visits followed the same procedures for questionnaire interviews, biospecimen collection, pQCT and DXA scans (340). A total of 1605 men completed the follow-up pQCT exam (80% of survivors). For the current analysis, we excluded men who were not of African ancestry (39 men) yielding an analytical cohort of 1,576. Men who did not return ($n = 377$) were significantly older, lighter, shorter and had low grip strength. Men who returned had significantly or nearly significantly greater age-adjusted TA, CA, CSMI, SM, and SSI as well as thicker cortices and larger PeriC and EndoC at the tibia. At the radius, TA and PERiC and EndoC were not different between men who returned and those who did not (data not shown). The Institutional Review Boards of the University of Pittsburgh and the Tobago Ministry of Health and Social Services approved this study and all participants provided written informed consent before data collection.

7.3.2 Peripheral QCT measures of cortical bone and adipose tissue at the tibia

pQCT scans were performed at the left tibia using the Stratec XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany) adhering to a standardized protocol for patient positioning and scanning at both the baseline and follow-up visits. A scout view was initially obtained prior to the baseline pQCT scan to define an anatomic reference line for the relative location of the subsequent scans at the tibia. Tibia length was measured from the medial malleolus to the medial condyle of the tibia. Scans were taken at 33% of the total length of tibia. Measurements at the 33% sites represent predominantly cortical bone. A single axial slice of 2.5 mm thickness with a voxel size of 0.5 mm and a speed of 20 mm/s was taken at all locations. Image processing was performed by a single investigator using the Stratec software package (Version 5.5E). All 33% tibia scans were analyzed using identical parameters for contour finding and separation of total and cortical bone (contour mode 2, T=169 mg/cm³; peel mode 2; Cortmode 1) to determine bone geometric properties. SSI was determined with Cortmode 1 (T = 280mg/cm³). CrtThk (mm), PeriC (mm), EndoC (mm), and polar SSI (SSI, mm³) were measured. It is not possible to estimate material properties like elastic modulus from QCT measurements. However, vBMD measured by QCT has an approximately linear relationship with elastic modulus. Based on this relationship, Schiessl *et al.* (382) developed the SSI, which is the integrated product of section modulus and vBMD normalized to the maximal physiological cortical vBMD of human bones. SM (mm³) was first calculated as $(a \times d^2)/d_{max}$, where a is the cross-sectional area of a voxel (mm²), d is the distance of the voxel from the center of gravity (mm), and d_{max} is the maximum distance or eccentricity of any voxel to the center of gravity (mm). The ratio of cortical BMD and normal physiological BMD (1200 mg/mm³) provides an estimate of the modulus of elasticity.

Scans taken at 66% of the total length of tibia measured TAT (mm^2), muscle cross-sectional area (mm^2), and muscle density (mg/cm^3), a validated measure of fatty infiltration of the muscle. (383) Muscle measures are obtained at the calf because this site had the largest circumference and associated with the least variability among individuals. (384) Intermuscular fat (IM) fat, which is fat found beneath the fascia lata, and muscle density, which reflects the amount of fat infiltration within the muscle cells and between individual muscle fibers. Lower muscle density corresponds to greater fat content in the muscle or greater intramuscular adipose tissue (IMAT) content. (385) Calf TAT area was computed as a sum of the SAT and visible NSAT. %SAT was calculated thus: $(\text{SAT}/\text{TAT}) \times 100$. %NSAT was also similarly calculated. In a pQCT scan, adipose tissue, muscle and bone can be distinguished from each other based on their mineral equivalent densities, which are $0 \text{ mg}/\text{cm}^3$, $80 \text{ mg}/\text{cm}^3$ and $1200 \text{ mg}/\text{cm}^3$ respectively. IMAT is detected when there is a shift in the mineral equivalent densities from 80 (indicative of muscle) to 0 (indicative of fat). Muscle density is calculated as a ratio of muscle mass (mg) and muscle area (cm^2). (386)

Coefficients of variation were determined for pQCT scans by replicating measurements on 15 subjects with $\text{CV} \leq 2.1\%$ for all measures. Daily phantom scans were analyzed to ensure long-term scanner stability.

7.3.3 Anthropometric measurements

Body weight was measured in kilograms with participants wearing light clothing and without shoes using a calibrated balance beam scale. Height was measured in centimeters without participants wearing shoes using a wall-mounted height board. Two height measurements were made and the average was used in analysis. BMI was calculated as weight in kilograms divided by

height in meters squared. Whole body lean mass was also measured using DXA (Hologic QDR 4500W; Hologic Inc., Bedford, MA).

7.3.3.1 Other measurements

Trained interviewers and nurses administered questionnaires to participants. We focused on potential correlates of bone strength and bone geometry based on the literature and from our previous findings of correlates for BMD in this cohort. (336) We collected information pertaining to demographic characteristics, medical history, physical inactivity, and lifestyle habits. Alcohol use was defined as having regularly consumed 4 or more drinks per week in the past 12 months. We also used hours of television watching per week as a surrogate of physical inactivity; the cut-off was set at 14 hours or more per week as a measure of sedentary behavior.

7.4 Statistical analysis

Measures of cortical BMD, SSI and structure were expressed as an annualized percentage rate of change from baseline (%/yr). %SAT and %NSAT were calculated as fractions of calf TAT. We assessed Spearman correlation coefficients for baseline weight, weight change since baseline, calf TAT and muscle density. Any covariate that is a continuous variable was expressed in terms of 1 SD change. We assessed age and calf TAT as well as weight and calf TAT interactions. Analysis of Covariance (ANCOVA) was used to obtain mean annualized percentage change in measures of BMD, SSI and geometry after multivariable adjustment. We assessed multicollinearity using VIF with a value of 10 or more suggesting multicollinearity. We fit four models: model 1 presented means (SE) adjusted for baseline age and baseline height; model 2

presented means adjusted for baseline age, baseline height, self-reported current smoking at baseline, self-reported alcohol consumption and self-reported television watching; model 3 presented means adjusted for change in body weight since baseline in addition to the covariates in model 2; lastly, model 4 presented means adjusted for baseline weight in addition to the covariates in model 3. We have presented adjusted means and standard errors. The analyses were repeated using all measures of calf fat as continuous variables, square root transformations for SAT, NSAT and calf TAT improved the distributions considerably. However, muscle density did not improve on transformation and was examined by quartiles. All statistical analyses were performed using the Statistical Analysis System (SAS, version 9.4; SAS Institute, Cary, NC).

7.5 Results

The mean age of the men included in our analytical sample was 56.8 ± 9.1 years (Table 10). Men experienced statistically significant changes in cortical BMD, SSI and bone structure at the tibia over the average 6.2 ± 0.5 years of follow-up (range 4.9 to 8.7 years) (Chapter 5.5). PeriC and EndoC increased significantly from baseline (Chapter 5.5). Overall, there were significant reductions in cortical BMD, CrtThk and SSI (Chapter 5.5). Average baseline TAT was $1810.4 \pm 770.2 \text{ mm}^2$ (Table 10) of which on average 75% was SAT.

Highly significant positive and negative correlations were observed between baseline measures of TAT and baseline body weight and baseline muscle density at the calf. Baseline TAT was not correlated with change in weight from baseline (Table 11).

Association between TAT and the rate of change in cortical BMD, SSI and structure at the tibia

Across increasing quartiles of TAT, greater cortical BMD loss and increased cortical thinning were observed which remained significant on multivariable adjustment for age, height, lifestyle factors, and weight change from baseline. However, after adjustment for baseline body weight, the association between calf TAT and rate of change in cortical thickness was no longer significant (Figure 10). On the other hand, the association between rate of change in cortical BMD loss was strengthened (Figure 10; $p < 0.001$). Change in SSI showed higher losses across increasing quartiles of calf TAT; this association was significant when adjusted for age, height, lifestyle factors and change in weight from baseline. However, it was no longer significant on adjusting for baseline weight. Rate of periosteal expansion increased across quartiles of calf TAT, the age and height-adjusted model was significant ($p\text{-value} < 0.05$); however further multivariable adjustment attenuated this association. Rate of endosteal expansion appeared to increase with increasing baseline calf TAT quartiles when adjusted for age, height and lifestyle. The statistically significant linear trend was lost upon adjustment for change in weight from baseline and baseline weight (Table 12). The results were consistent when TAT was studied as a continuous variable (data not shown). Further, the results were similar when baseline total body lean mass measured by DXA was used to adjust for mechanical loading instead of baseline total body weight (data not shown).

Association between %SAT and the rate of change in cortical BMD, SSI and geometry at the tibia

Rate of loss in cortical BMD was lower across ascending quartiles of %SAT. This association was significant (Table 13; p for trend < 0.01 for all) in all four models. There was no discernible pattern in the rate of cortical thinning and rate of loss in SSI across quartiles of %SF. Endosteal expansion increased across ascending quartiles of %SF after adjusting for baseline age and baseline height (Table 13; p for linear trend < 0.05). However, on further multivariable

adjustment, this association was no longer significant. Rate of periosteal expansion increased across quartiles of %SAT and statistically significant in every model (Table 13; p for trend < 0.05 for all). The results were consistent when examined across quartiles of baseline SAT area at the calf (data not shown).

Reciprocal relationships (reciprocal to %SAT) with %NSAT were seen with rate of change in cortical BMD (data not shown) and rate of endosteal expansion (data not shown). Similar to the association with %SAT, rates of cortical thinning and change in SSI did not show any NSAT-related patterns. Rate of periosteal expansion declined across ascending quartiles of %NSAT; however, the association was no longer significant for trend upon additional adjustment for change in weight from baseline and baseline weight.

Association between muscle density at the calf and the rate of change in cortical BMD, SSI and structure at the tibia

Across increasing quartiles of muscle density at the calf, an indicator of less fatty infiltration of the muscle, rate of loss in SSI was significantly lower (Table 14; $p < 0.05$ for models 1 to 3). However, additionally adjusting for baseline body weight attenuated this association ($p = 0.45$). Higher muscle density was associated with a slower rate of cortical BMD, but was only significant after adjusting for baseline weight in Model 4 (Table 14; p -value for trend = 0.01).

7.6 Discussion

To our knowledge this is the first study to investigate rates of change in cortical bone density, strength and structure in relation to baseline ectopic fat depots in men of African ancestry. We found that higher TAT at the calf was associated with greater losses in cortical BMD. On

examining the SAT and NSAT compartments of calf fat, decreasing and increasing rates of cortical BMD loss were observed respectively, which remained significant on multivariable adjustment, including baseline body weight — a surrogate for mechanical loading. Across increasing quartiles of %SAT, greater periosteal expansion was observed, which was materially unaltered on multivariable adjustment in all four models. In addition, we also examined the relationship between baseline muscle density at the calf and rates of change in cortical bone measures of density, strength and geometry, which showed significantly lower rates of loss in cortical BMD after adjusting for the mechanical loading effect of body weight. The lower rate of loss in SSI was no longer significant after adjusting for baseline body weight.

A highlight of our analyses is the relationship between rate of periosteal expansion and %SAT at the calf; greater periosteal expansion was seen across increasing quartiles of %SAT fat, and was significant for linear trend in all four models. Changes in the periosteal circumference have not been consistently examined in epidemiologic studies even though it is the only envelope, where bone formation continues to occur through life. We found a protective action of SAT on periosteal expansion that was independent of the mechanical loading effect of body weight. These findings suggest that periosteal expansion could potentially be regulated by cytokines and/or hormones secreted by SAT.

Zhao and colleagues (302) examined the relationship of obesity and bone mass excluding the mechanical loading effects of body weight. They used DXA-measures of bone mass and fat mass from two large samples of men and women of Caucasian and Chinese ethnicities having average ages of 62 and 27 respectively. They reported that fat mass by itself did not have a protective effect. In our analyses we found similar results; rate of loss in cortical BMD was higher across ascending quartiles of calf TAT suggesting mechanisms other than mechanical loading.

However, we found that the relationship between calf TAT and losses in SSI were not independent of the mechanical loading effect of body weight.

Gilsanz and colleagues used QCT-measured bone strength, SAT, VAT, and muscle area in a sample of 100 young women aged 15 to 25. They reported that VAT and SAT had negative and positive associations respectively with femoral bone strength after adjustments for limb length and muscle cross-sectional area — a surrogate for mechanical loading. (303) Our analyses also revealed reciprocal relationships between calf fat compartments and rates of loss in cortical BMD.

Ng and colleagues (305) examined a variety of bone quality using a combination of DXA, QCT and HR-pQCT in men and women aged 21 to 97 years. In older men, significant positive correlations were reported between total body fat mass, SAT and VAT with cortical thickness, cortical area at the ultra-distal radius, and trabecular microarchitecture at the ultra-distal radius. All these positive correlations were lost upon adjustment for age, bioavailable E2, height and particularly weight. While our study is limited by the availability of measure of cortical and trabecular microarchitecture, we did not find significant associations between TAT or its components and rates of cortical thinning on full adjustment. Furthermore, an examination of rates of trabecular BMD loss at the distal tibia showed no association with TAT (data not shown).

Even though individuals of African ancestry have lower total body fat (387) and visceral fat (388) compared to Caucasians, they have higher levels of skeletal muscle fat infiltration. (389) Fat infiltration of skeletal muscle may play a role in loss of mobility (390,391) and decreased muscle strength. (392,393) Our results showed that higher muscle density (indicative of less intra and inter-muscular fat infiltration) was associated with lower rates of cortical BMD loss, only after adjusting for mechanical loading effect of body weight. On the contrary, the association between higher muscle density and rate of change in SSI disappeared after adjusting for body weight. These

findings are pertinent because there is evidence linking intramuscular fat with elevated risk of all-cause mortality independent of general body fat (394) and incident T2D. (395)

We examined muscle density at the calf, which is a valid (383) and reliable (385) measure of lipid infiltration of muscle cells, and therefore a surrogate of intramuscular fat. It has been shown that men of African ancestry have higher intramuscular lipid infiltration independent of total adiposity. (396) We found less fatty and more dense muscle to be associated with lower rates of cortical BMD loss even after adjusting for mechanical loading. The mechanisms linking muscle density and BMD likely involve the release of local factors like IL6, IGF-1, fibroblast growth factor -1 (FGF-1) and myostatin, which directly affect bone cells. (397)

Even though we assessed TF at the calf, it only represents a relatively small depot of total body fat. However, calf fat has been shown to be highly correlated with CT-measured thigh fat ($r=0.62$, $p<0.01$) (385); furthermore, the proximity of the anatomical sites for adipose tissue and cortical bone measurements is a potential strength. Our study findings may be limited by potential recall bias from questionnaire assessments of lifestyle factors; volunteer bias arising from our participants, who might differ from the general population. Nonetheless, our study also has several strengths including a relatively large sample size, longitudinal study design, pQCT-derived measures of bone strength and bone geometry, and a wealth of data on potential covariates. Moreover, the present study addresses a major gap in the literature on the association between fat distribution and age-related changes in bone, particularly among black men who have been under-represented in past studies. Additional studies investigating diverse ectopic fat depots are needed to better understand the physiological mechanisms underlying skeletal changes with aging in black men.

In conclusion, our study demonstrates that higher total fat area at the calf is associated with increased rates of cortical bone mineral density loss. We also found a greater percentage of subcutaneous fat at the calf was associated with greater rates of periosteal expansion independent of body weight, suggesting that the underlying biological pathway likely involves factors other than mechanical loading. In contrast, we found that the detrimental effects of higher total fat at the calf on rates of change in torsional bone strength were not independent from the mechanical loading effect of baseline body weight, suggesting that bone torsional strength is maintained by periosteal expansion. Lastly, we found that less fatty and more dense calf muscle was associated with decreased rates of cortical bone mineral density loss, highlighting the importance of maintaining healthy muscle with older age. These findings underscore the need to further study the relationship between adipose tissue distribution and skeletal health as in the coming decades obesity and osteoporosis will likely coexist.

7.7 Tables and Figures

Table 10 Baseline characteristics of Tobago Men

Baseline characteristic (n = 1576)	Mean/%	Std Dev/#
Age (yr)	56.88	9.14
Height (cm)	175.47	6.89
Body Weight (kg)	84.97	14.81
BMI	27.54	4.41
Total calf fat (mm ²)	1810.40	770.29
Total non-subcutaneous adipose tissue at calf(mm ²)	404.07	235.84
Total subcutaneous adipose tissue at calf (mm ²)	1383.82	666.34
Percentage subcutaneous adipose tissue at calf (%)	75.13	14.24
Percentage non-subcutaneous adipose tissue at calf (%)	24.87	14.24
Muscle density (mg/cm ³)	73.62	3.82
Hypertension	48.83	769
Diabetes	17.09	261
Any Fracture	21.22	327
Current smoking	10.47	164
4 or more drinks/week	10.35	162
TV watching > 14 hours/week	38.42	599

BMI-Body Mass Index; CVD-

Table 11 Spearman correlation coefficients for baseline anthropometric measures.

	Total calf fat	Muscle density at calf	Body weight	Weight change from baseline
Total calf fat	1.00			
Muscle density at calf	-0.50***	1.00		
Body weight	0.65***	-0.32***	1.00	
Weight change from baseline	-0.03	0.06*	-0.13***	1.00

*p-value<0.05, *** p-value < 0.001

Table 12 Rates of change in cortical bone measures by total calf fat

(%/yr)		TAT				
Cortical density		Quartile 1 (n = 390)	Quartile 2 (n = 385)	Quartile 3 (n= 384)	Quartile 4 (n= 384)	p-value for linear trend
	Model 1	-0.26 (0.01)	-0.25 (0.01)	-0.25 (0.01)	-0.28 (0.01)	0.04
	Model 2	-0.26 (0.01)	-0.25 (0.01)	-0.25 (0.01)	-0.28 (0.01)	0.04
	Model 3	-0.26 (0.01)	-0.25 (0.01)	-0.25 (0.01)	-0.29 (0.01)	0.05
	Model 4	-0.24 (0.01)	-0.24 (0.01)	-0.26 (0.01)	-0.31 (0.01)	<0.0001
Cortical thickness						
	Model 1	-0.52 (0.03)	-0.54 (0.03)	-0.53 (0.03)	-0.59 (0.03)	0.08
	Model 2	-0.52 (0.03)	-0.54 (0.03)	-0.53 (0.03)	-0.60 (0.03)	0.07
	Model 3	-0.52 (0.03)	-0.54 (0.03)	-0.53 (0.03)	-0.60 (0.03)	0.07
	Model 4	-0.51 (0.03)	-0.54 (0.03)	-0.53 (0.03)	-0.61 (0.03)	0.07
Periosteal Circumference						
	Model 1	0.22 (0.01)	0.23 (0.01)	0.24 (0.01)	0.25 (0.01)	0.03
	Model 2	0.22 (0.01)	0.23 (0.01)	0.24 (0.01)	0.25 (0.01)	0.09
	Model 3	0.23 (0.01)	0.23 (0.01)	0.24 (0.01)	0.24 (0.01)	0.16
	Model 4	0.23 (0.01)	0.23 (0.01)	0.24 (0.01)	0.24 (0.01)	0.49
Endosteal circumference						
	Model 1	0.86 (0.04)	0.88 (0.04)	0.90 (0.04)	0.98 (0.04)	0.02
	Model 2	0.87 (0.04)	0.87 (0.04)	0.90 (0.04)	0.98 (0.04)	0.05
	Model 3	0.88 (0.04)	0.88 (0.04)	0.90 (0.04)	0.97 (0.04)	0.08
	Model 4	0.88 (0.04)	0.88 (0.04)	0.89 (0.04)	0.97 (0.04)	0.18
SSI						
	Model 1	-0.43 (0.05)	-0.51 (0.05)	-0.51 (0.05)	-0.73 (0.05)	0.0001
	Model 2	-0.43 (0.05)	-0.51 (0.05)	-0.52 (0.05)	-0.72 (0.05)	0.0002
	Model 3	-0.42 (0.05)	-0.51 (0.05)	-0.52 (0.05)	-0.70 (0.05)	0.0004
	Model 4	-0.49 (0.05)	-0.54 (0.05)	-0.51 (0.05)	-0.62 (0.05)	0.23

Model 1: Baseline age + Baseline height; Model 2: Model 1 + current smoking, 4 or more alcoholic drinks in a week, and TV watching; Model 3: Model 2 + percentage weight change from baseline; Model 4: Model 3 + baseline weight. Quartile 1: < 1273.00 mm²; Quartile 2: 1273 – 1712.25 mm²; Quartile 3: 1712.25 - 2232.50 mm²; Quartile 4: > 2232.50 mm².

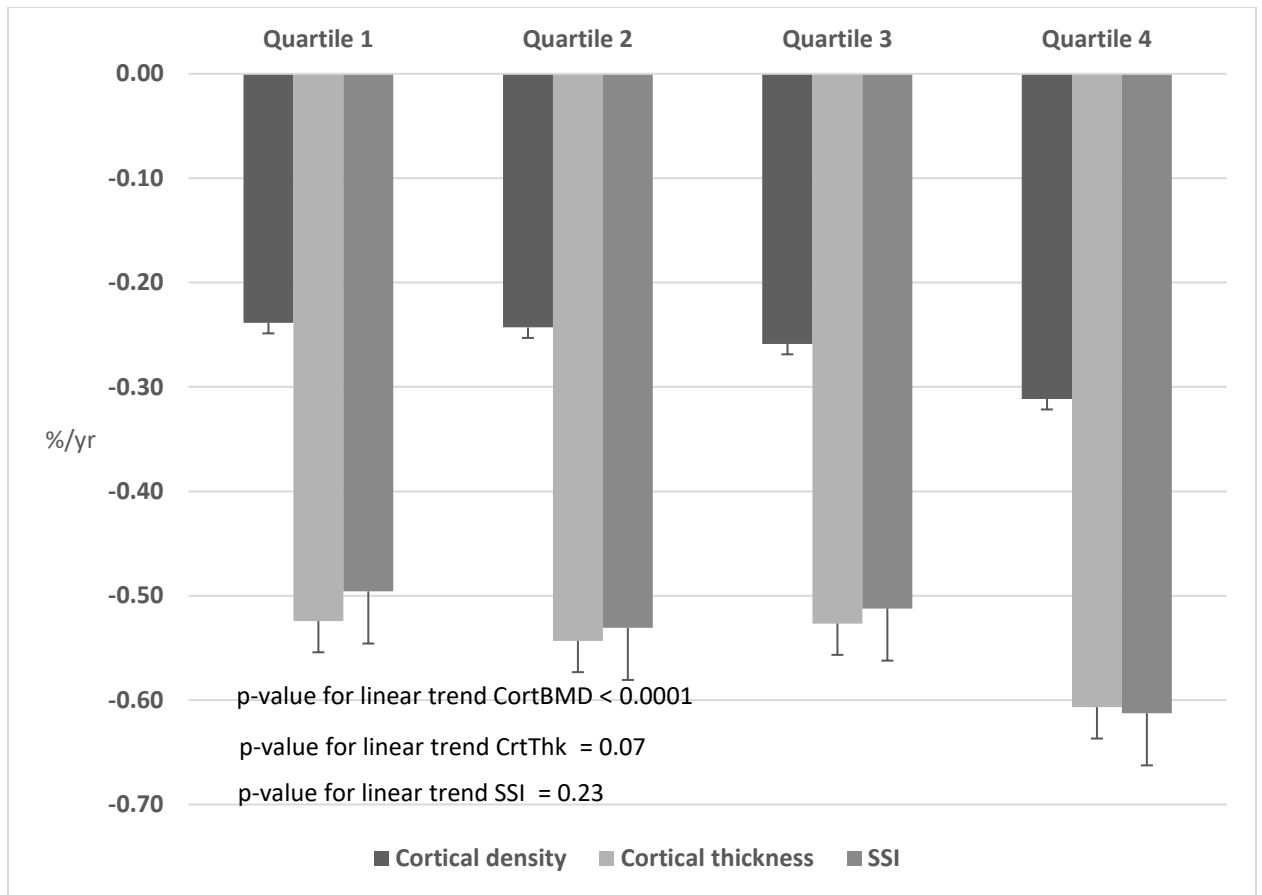


Figure 10 Rates of change in cortical bone measures by quartiles of total calf fat

Adjusted for age, height, baseline weight, percentage weight change, smoking, alcohol consumption and TV watching. Quartile 1: < 1273.00 mm²; Quartile 2: 1273 – 1712.25 mm²; Quartile 3: 1712.25 - 2232.50 mm²; Quartile 4: > 2232.50 mm².

Table 13 Rates of change in change in cortical bone measures by quartiles of %SAT at the calf

(%/yr)		%SAT				
		Quartile 1 (n =390)	Quartile 2 (n=384)	Quartile 3 (n=384)	Quartile 4 (n=385)	p-value for linear trend
Cortical density	Model 1	-0.28 (0.01)	-0.28 (0.01)	-0.25 (0.01)	-0.24 (0.01)	0.002
	Model 2	-0.28 (0.01)	-0.28 (0.01)	-0.25 (0.01)	-0.24 (0.01)	0.002
	Model 3	-0.28 (0.01)	-0.28 (0.01)	-0.25 (0.01)	-0.24 (0.01)	0.001
	Model 4	-0.28 (0.01)	-0.28 (0.01)	-0.25 (0.01)	-0.24 (0.01)	0.002
Cortical thickness	Model 1	-0.53 (0.03)	-0.54 (0.03)	-0.54 (0.03)	-0.56 (0.03)	0.47
	Model 2	-0.53 (0.03)	-0.54 (0.03)	-0.54 (0.03)	-0.56 (0.03)	0.57
	Model 3	-0.54 (0.03)	-0.55 (0.03)	-0.53 (0.03)	-0.56 (0.03)	0.86
	Model 4	-0.54 (0.03)	-0.55 (0.03)	-0.53 (0.03)	-0.55 (0.03)	0.93
Periosteal Circumference	Model 1	0.21 (0.01)	0.23 (0.01)	0.25 (0.01)	0.25 (0.01)	0.0045
	Model 2	0.21 (0.01)	0.23 (0.01)	0.25 (0.01)	0.25 (0.01)	0.01
	Model 3	0.21 (0.01)	0.23 (0.01)	0.24 (0.01)	0.25 (0.01)	0.02
	Model 4	0.21 (0.01)	0.23 (0.01)	0.24 (0.01)	0.25 (0.01)	0.02
Endosteal circumference	Model 1	0.82 (0.04)	0.93 (0.04)	0.92 (0.04)	0.95 (0.04)	0.04
	Model 2	0.83 (0.04)	0.93 (0.04)	0.91 (0.04)	0.95 (0.04)	0.08
	Model 3	0.83 (0.04)	0.94 (0.04)	0.90 (0.04)	0.94 (0.04)	0.11
	Model 4	0.84 (0.04)	0.94 (0.04)	0.90 (0.04)	0.94 (0.04)	0.15
SSI	Model 1	-0.53 (0.05)	-0.55 (0.05)	-0.54 (0.05)	-0.53 (0.05)	0.98
	Model 2	-0.53 (0.05)	-0.56 (0.05)	-0.54 (0.05)	-0.53 (0.05)	0.88
	Model 3	-0.53 (0.05)	-0.56 (0.05)	-0.54 (0.05)	-0.51 (0.05)	0.68
	Model 4	-0.56 (0.05)	-0.57 (0.05)	-0.52 (0.05)	-0.49 (0.05)	0.26

Model 1: Baseline age + Baseline height; Model 2: Model 1 + current smoking, 4 or more alcoholic drinks in a week, and TV watching; Model 3: Model 2 + percentage weight change from baseline; Model 4: Model 3 + baseline weight.

Table 14 Rates of change in change in cortical bone measures by quartiles of muscle density

(%/yr)						
Cortical density		Quartile 1 (n = 390)	Quartile 2 (n = 385)	Quartile 3 (n= 384)	Quartile 4 (n= 384)	p-value for linear trend
	Model 1	-0.26 (0.01)	-0.27 (0.01)	-0.28 (0.01)	-0.24 (0.01)	0.21
	Model 2	-0.26 (0.01)	-0.27 (0.01)	-0.28 (0.01)	-0.24 (0.01)	0.16
	Model 3	-0.26 (0.01)	-0.27 (0.01)	-0.28 (0.01)	-0.23 (0.01)	0.11
	Model 4	-0.27 (0.01)	-0.27 (0.01)	-0.28 (0.01)	-0.22 (0.01)	0.01
Cortical thickness						
	Model 1	-0.57 (0.03)	-0.56 (0.03)	-0.52 (0.03)	-0.52 (0.03)	0.09
	Model 2	-0.57 (0.03)	-0.56 (0.03)	-0.52 (0.03)	-0.52 (0.03)	0.10
	Model 3	-0.57 (0.03)	-0.55 (0.03)	-0.52 (0.03)	-0.52 (0.03)	0.10
	Model 4	-0.57 (0.03)	-0.55 (0.03)	-0.52 (0.03)	-0.52 (0.03)	0.13
Periosteal Circumference						
	Model 1	0.22 (0.01)	0.26 (0.01)	0.23 (0.01)	0.23 (0.01)	0.49
	Model 2	0.23 (0.01)	0.25 (0.01)	0.23 (0.01)	0.23 (0.01)	0.56
	Model 3	0.23 (0.01)	0.25 (0.01)	0.23 (0.01)	0.23 (0.01)	0.75
	Model 4	0.22 (0.01)	0.25 (0.01)	0.23 (0.01)	0.24 (0.01)	0.81
Endosteal circumference						
	Model 1	0.92 (0.04)	0.98 (0.04)	0.88 (0.04)	0.84 (0.04)	0.08
	Model 2	0.92 (0.04)	0.97 (0.04)	0.88 (0.04)	0.85 (0.04)	0.10
	Model 3	0.92 (0.04)	0.96 (0.04)	0.88 (0.04)	0.85 (0.04)	0.14
	Model 4	0.91 (0.04)	0.96 (0.04)	0.88 (0.04)	0.96 (0.04)	0.24
SSI						
	Model 1	-0.61 (0.05)	-0.52 (0.05)	-0.57 (0.05)	-0.41 (0.05)	0.02
	Model 2	-0.61 (0.05)	-0.52 (0.05)	-0.58 (0.05)	-0.41 (0.05)	0.03
	Model 3	-0.61 (0.05)	-0.52 (0.05)	-0.57 (0.05)	-0.41 (0.05)	0.03
	Model 4	-0.56 (0.05)	-0.51 (0.05)	-0.58 (0.05)	-0.47 (0.05)	0.45

Model 1: Baseline age + Baseline height; Model 2: Model 1 + current smoking, 4 or more alcoholic drinks in a week, and TV watching; Model 3: Model 2 + percentage weight change from baseline; Model 4: Model 3 + baseline weight.

8.0 Overall Discussion and Public Health Importance

This dissertation described patterns of age-related cortical bone loss and its determinants in middle-aged and older African ancestry men. It examined the link between biomarkers of inflammation and rates of change in cortical bone measures, as well as, the relationship between regional depots of fat and rates of change in cortical bone measures. In particular, the results from this last area of research challenge the long-held belief in a positive association between adiposity and the skeleton.

8.1 Overall Discussion

Paper 1 showed that endosteal expansion overtook periosteal expansion with advancing age, such that there was net cortical thinning. However, SSI at the radius and tibia was largely maintained and did not show significant age-related declines across age-groups even when adjusted for independent correlates, reflecting the physiological adaptation of the skeleton by way of periosteal expansion to maintain bone strength. Previously, endosteal expansion outpacing periosteal expansion had been demonstrated in primarily cross-sectional studies, with only one other longitudinal study revealing gross underestimates of the rates of bone loss reported in cross-sectional studies. While, the estimates of rates of change in cortical bone structure and strength from paper 1 were consistent with extant reports, it is to be noted that the skeletal sites studied have not been consistent across reports. (105,106)

In paper 2, there was no consistency across the associations between inflammation biomarkers and cortical bone measures in cross-sectional and longitudinal findings. Contrary to the working hypothesis, higher levels of IL6 were associated with slower rates of cortical BMD loss. The findings could partly be attributed to some methodological limitations of the approach, viz. using a single measure of IL6. However, in light of the inconsistent findings from studies examining this very association, these results highlight the gaps in the current understanding of biomarkers of inflammation and their skeletal effects. HsCRP, on the other hand, was not a predictor of cortical bone loss in cross-sectional or longitudinal analyses. Some studies examining the relationship between inflammation markers and fractures reported an increased risk of fracture with elevated levels of inflammation markers independent of BMD. (263–265,267) The findings from this dissertation suggest that inflammation marker-related perturbations, if any, likely occur at the microarchitectural level and would necessitate HR-QCT assessments. However, Rolland *et al.* did not report an association between markers of inflammation and HR-QCT-measured cortical microarchitecture. (275)

In paper 3, after statistically adjusting for the mechanical-loading effect of body weight, having higher TAT at the calf was associated with significantly greater annual losses in cortical BMD at the tibia. When the total fat compartment was further examined by its subdivisions — SAT and NSAT areas — they were associated with slower and faster rates of cortical BMD loss respectively. Furthermore, having higher %SAT at the calf was associated with significantly greater periosteal expansion even after adjusting for the biomechanical loading effect of body weight. When muscle density, a valid surrogate for lack of intramuscular fat, was examined: higher muscle density was associated with significantly slower loss of cortical BMD only after adjusting for mechanical loading, whereas loss of SSI was no longer significant after adjusting for

mechanical loading, suggesting that in these middle-aged and older African ancestry men, the skeletal adaptations to preserve bone strength — periosteal expansion increasing total bone area and consequently bone bending strength — had not yet been overcome. Together, the results suggest that in the face of ongoing age-related cortical thinning, higher ectopic fat (assessed here as non-subcutaneous fat and lower muscle density) potentially disrupts age-related periosteal expansion, which could interfere with the ability of the skeleton to redistribute available bone material to confer maximum mechanical advantage.

8.2 Strengths and Limitations

Approximately 60% of all age-eligible men on the island participated in the Tobago Bone Health Study. While this recruitment exceeds the usual percent representation of most epidemiologic studies of bone health, the sample is still subject to volunteer bias. While not the main exposures of interest, many of the medical history variables were self-reported and, therefore, subject to recall bias. In addition to self-report, variables such as diabetes and hypertension status were based on objective measures of BP and blood glucose, as well as, history of medication for each condition. Due to low admixture in the population, the findings may have limited generalizability to African-Americans, who generally have 20% admixture from non-African ancestry (398); however, this very admixture makes studies in African-Americans challenging due to confounding from non-African ancestry. One potential limitation arises from a selection bias as the analyses are limited to men, who were healthy enough to complete two pQCT scans. Inevitable losses to follow-up were due to death or inability of the participant to travel to complete the study visits; however, the retention rates were high as compared to other similar epidemiologic studies.

Those who were lost to follow-up were generally older and had poorer health as compared to those who successfully completed the follow-up study visit. The characteristics of these men are summarized in the appendices.

Important strengths of this longitudinal study include a large sample size of an understudied population, pQCT measures of bone density, strength and geometry fat at two time points, and a wealth of covariates and data on potential confounders.

8.3 Public Health Importance

Of the 2 million fractures with direct costs nearing \$17 billion in the year 2005, 73% occurred at nonvertebral sites accounting for 94% of the costs; 29% of these fractures occurred in men; 61% of the fractures occurred in those 65 and older, who accounted for 81% of the total costs. The fracture burden in the non-white population in the United States is projected to increase by the year 2025; the black population has been projected to see a 79% increase from 2005 to 2025 in terms of fracture costs. (40) Despite these dire projections, there is a paucity of information on skeletal aging in African ancestry men. Furthermore, results obtained from studies in African Americans may be confounded by European ancestry. (221) However, Tobagonians are predominantly of Western African ancestry, so the Tobago Bone Health Study is able to address the important knowledge gap of a lack of studies on skeletal aging in African ancestry populations. (30–36)

The key aspect of this dissertation with public health relevance is its focus on bone structure and measures of strength in the cortical compartment as it is not merely the amount of bone mass, but bone structure that determines bone strength. It is important to study bones by modeling them

as structures to ensure that assessments of bone strengths at various sites do not represent transient changes. (399) However, as population-scale modeling of whole bones as structures is cumbersome and expensive, surrogates like bone structure measures from peripheral skeletal sites can provide a wealth of information about the contributions of bone structure and its changes to whole bone strength. Investigators have expressed concern that skeletal research has focused exclusively on trabecular bone often overlooking the larger cortical component as merely a protective shell. (400) The vital role of cortical bone in its contribution to bone strength has been underscored in a simulation study, wherein the entire radius was digitally reconstructed with micro-CT and 20% bone loss was simulated by: reducing cortical thickness, reducing trabecular numbers, and reducing trabecular number or thinning in both compartments. The reductions in bone strength was nearly 40% in the cortical-only model and approximately 10% in the predominantly trabecular or combined models. (401)

Also, of particular public health relevance was a detailed examination of the periosteal envelope and its correlates, which represents a potential target for pharmacological and non-pharmacological intervention. The magnitude of periosteal bone formation and its determinants have rarely been described in adult populations, (111) even though this envelope continues to expand and adapt throughout life, and the skeleton's ability to preserve mechanical integrity are attributed to periosteal bone deposition. This dissertation research found that the use of beta-blocking drugs was independently associated with decreased periosteal expansion at the tibia, an unexpected association because studies have found that propranolol, a non-selective beta-blocking drug, increased the rate of bone formation and the number of osteoblasts in rat models, (358) and use of beta-blocking agents has been associated with a reduced risk of fracture. (78) Another commonly-used class of drugs associated with expansion at the periosteal envelope were NSAIDs,

whose use was associated with increased rate of periosteal expansion at the radius. Also, of particular importance, in this African ancestry population with a high prevalence of hypertension, particularly untreated hypertension, (362) was that the use of thiazide diuretics was associated with lower rates of cortical thinning and endosteal expansion. A positive association between thiazide use and BMD has been consistently reported, whereas the associations with other drugs, like beta-blocking drugs and calcium-channel blockers have been inconsistent. (402) Considering thiazide diuretics are frequently the drug of choice for starting anti-hypertension therapy, efforts should be made to identify and treat hypertensive men in this population.

Since osteoporosis-related fractures are preventable, it is essential to understand the pathogenesis of age-related bone loss. The results from this dissertation research do not support the notion that osteoporosis arises from an inflammation-related process; however, the results were limited by the use of a single measure of inflammation markers.

The prevalence of obesity (defined by BMI) has been on the rise, and has been increasingly documented since the 1990s. (372,373) Based on data from National Health and Nutritional Examination Surveys (NHANES) from years 1999 – 2010, the overall age-adjusted prevalence rate of obesity among men was 35.5% (95% CI, 31.9%-39.2%) and ranged from 36.2% (95% CI, 31.8%-40.8%) among non-Hispanic white men to 38.8% (95% CI, 33.9%-43.9%) among non-Hispanic black men. When combined with the prevalence of overweight (defined by BMI), the rates are even more alarming: nearly 73.9% (95% CI, 70.0% -77.8%) among men. (372) In this dissertation research, having higher calf fat area, particularly non-subcutaneous calf fat was detrimental to cortical BMD independent of total body weight. There is evidence linking intramuscular fat, assessed by muscle density attenuation, with elevated risk of all-cause mortality independent of general body fat in African ancestry men. (394) Moreover, intramuscular fat

explains the observed race/ethnic differences in susceptibilities for developing T2D. (395) In light of these, the current findings regarding ectopic fat and bone loss have important implications for the development and management of weight loss strategies. As such, it is crucial for us to have a better understanding of ectopic fat infiltration of the muscle in order for it to be a modifiable risk factor.

In conclusion, this dissertation work has addressed the scarcity of information pertaining to skeletal health in African ancestry men, as well as, information on age-related changes in cortical bone structure in aging men in general. Further, the unexpected positive association found between inflammation and bone loss in terms of density and strength highlight the complexity of the underlying biological mechanisms. Lastly, in examining the relationship between adiposity, particularly ectopic fat depots, and cortical bone health, this dissertation has attempted to evaluate the interplay between two body composition disorders – obesity and osteoporosis – that often coexist and are expected to become increasingly prevalent in coming years.

9.0 Future Directions

Future work to gain a deeper understanding of skeletal aging in African ancestry men should ideally include men younger than 40 years of age to better understand the timing and factors that facilitate endosteal resorption to overtake periosteal bone formation. The results in this dissertation from middle-aged men have not shown a significant age-related decline in SSI at the radius or tibia, when examined by age-groups, indicating that periosteal bone formation is continuing to preserve bone strength. However, it has been shown that loss in muscle accelerates after age 60, (403) thus affecting the largest source of bone strain to maintain mechanical loading. (48,404) Further, aging-related decreased physical activity, decreased sex hormones, decreased protein intake, and increased adipose tissue infiltration of muscle are associated with decreased muscle mass, strength and power. (405) Therefore, it is equally important to study large cohorts of men over 70 years of age to report and assess the impact of cortical bone losses.

In the absence of hallmark genetic causes, osteoporosis is a multifactorial disorder underscoring the importance of characterizing risk factors. Like many chronic conditions, the etiology for osteoporosis likely has many environmental contributors. The contribution of modifiable risk factors, such as diet and exercise, to skeletal health is important to design recommendations and interventions for maintaining skeletal health. Unfortunately, one of the most challenging risk factors to accurately collect data on is diet, not only because of reliability concerns, but also due to issues of validity. Similarly, reliable measures of physical activity are often not captured in epidemiologic studies. However, well-designed clinical trials assessing the efficacy of physical activity in preventing bone loss have been conducted in women, particularly post-menopausal women, (406–413) and have shown that a combination of high-impact exercise

and moderate to high-intensity resistance training is most effective in preventing age-related bone loss, (409–411,413,414) highlighting the need for similar studies in men.

Muscle force is the strongest voluntary load acting on the bone (48,404); higher bone strength and bone mass are associated with greater lean mass and muscle force in QCT and DXA studies. (415) These findings are the underlying rationale for studying bone and muscle as a unit, such as bone-muscle indices, and not in isolation. Even though, most of the variation in these indices is explained by DXA-measured BMD, (415) their study could potentially improve the current understanding of skeletal pathophysiology.

Lastly, the relationships between other ectopic fat depots and bone structure and strength at trabecular and cortical sites should be examined in combination with a deeper understanding of the natural history of changes in ectopic fat depots with age.

In conclusion, more longitudinal studies using imaging modalities like QCT of African ancestry men ranging from young to old with more objective assessments and repeated measures of covariates and serum biomarkers are warranted.

Appendix A Tobago Bone Health Study

The specific aims of this dissertation were addressed using data from the Tobago Bone Health Study.

Tobago, a part of Trinidad and Tobago, is an island measuring 7×26 miles in the Caribbean archipelago. Healthcare in Tobago is largely administered by the government and some private providers; there are 19 neighborhood health centers and one hospital overseen by the Tobago Regional Health authority; when required, residents seek higher-level of care in Trinidad under this system. (335)

Between 1997 and 2003, 3,170 men aged 40 and older were recruited for population-based prostate cancer screening for the first time on the island of Tobago, Trinidad & Tobago. (335) To be eligible, men had to be ambulatory, non-institutionalized and not terminally ill. Recruitment was achieved by means of public service announcements, flyers, information diffused by physicians and other healthcare workers, public presentations by urologists and oncologists, and mostly word of mouth. Approximately 60% of all age-eligible men on the island participated and participation was representative of the island parishes. The recruited cohort was 97% African, 2% East Indian, <1% white, and <1% "other" as defined by participant-report of paternal and maternal grandparents' ethnicity. Studies using molecular markers have confirmed the low non-African admixture (about 6%) in this population. (336)

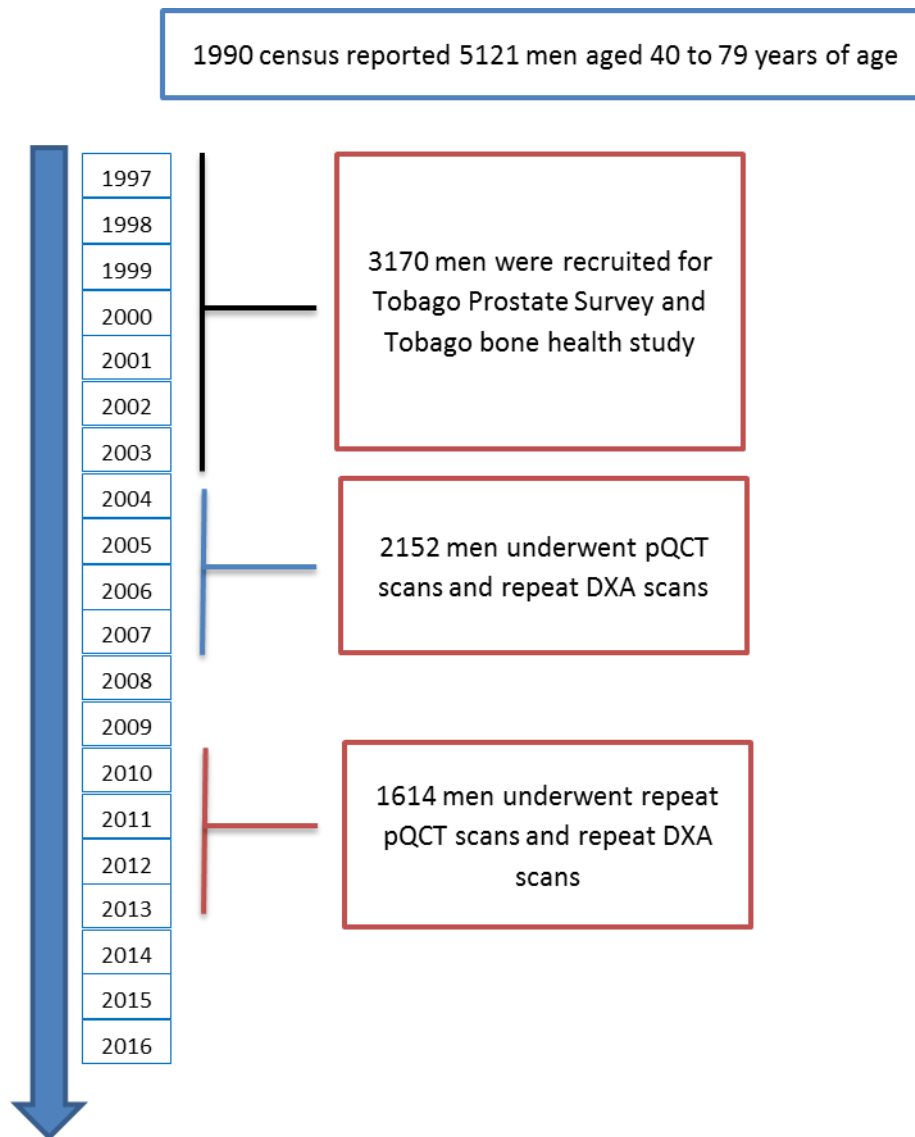


Figure A Overview of the TobagoBone Health Study

Table A Baseline characteristics of survivors and deceased

Baseline characteristics (N = 2099)	Mean (SD) or n (%)		
	Survivors n= 2000	Deceased n = 99	p-value *
Age (yrs)	58.31	69.33	<.0001
Weight (kg)	84.78	78.49	<.0001
Height (cm)	175.20	172.80	0.00
BMI	27.57	26.26	0.00
Grip Strength (kg)	43.25	33.74	<.0001
Hypertension (Y/N)	1013 (50.90)	88 (67.69)	0.03
Diabetes (Y/N)	372 (19.68)	50 (40.98)	<.0001
Myocardial Infarction	19 (0.97)	2 (2.11)	0.25
Stroke (Y/N)	38 (1.93)	3 (3.16)	0.43
Kidney disease (Y/N)	7 (0.36)	2 (2.13)	0.06
Cancer (Y/N)	150 (7.63)	24 (25.26)	<.0001
Current smoking (Y/N)	202 (10.30)	16 (12.70)	0.39
Alcohol consumption > 4 times a week in the last 12 months (Y/N)	209 (10.66)	13 (10.32)	0.90
Walking > than 3 times a week (Y/N)	1686 (81.02)	106 (5.09)	0.51
Walking in the past 7 days (Y/N)	1212 (61.87)	69 (55.20)	0.14
Osteoporosis (Y/N)	36 (1.84)	5 (5.32)	0.04
Any fracture (Y/N)	389 (20.12)	21 (16.80)	0.37
ADT (Y/N)	80 (4.11)	19 (15.32)	<.0001
Self-reported good health Excellent or good/ Fair, poor, very poor	1800 (92.45)	97 (78.23)	<.0001
3 or more chronic diseases (Y/N)	112 (5.56)	23 (17.42)	<.0001

*p-value for comparisons between deceased and survivors using t-test, Chi-square test, and Fisher's Exact test as applicable

Appendix B Stress-Strain Curve

Stress is defined as force per unit area. It may be compressive, tensile, or shear depending on the manner of loading. Strain is defined as percentage change in length or relative deformation. When plotted against each other, the stress-strain curve can be divided into an elastic region and a plastic region. The elastic and plastic regions are divided by the yield point, a point beyond which stresses cause permanent damage to the bone structure resulting in plastic deformation; this is also the point where the relationship between stress and strain is no longer linear. The elastic region is also referred to as pre-yield point and the plastic region is referred to the post-yield region. The slope of the relationship between stress and strain in the elastic region is called Young's modulus or elastic modulus, a measure of intrinsic stiffness of the material. Crack growths, trabecular microfracture, or their combinations are examples of post-yield strains. The area under the stress-strain curve is the toughness of the material or the energy required to cause fracture. The stress at which bone breaks is called breaking strength; the maximum stress a bone can sustain is called ultimate strength. With repetitive loading, mechanical properties like strength and Young's modulus degrade and fatigue ensues. In bone, repetitive loading leads to the appearance of cracks that coalesce leading to failure (Figure B).

Three stages characterize the fatigue life of a bone: crack initiation and accumulation; crack growth, and crack coalescence. The stage of micro cracking is rather rapid as is the stabilization; these cracks propagate in such a manner as to relieve stress concentrations extending the fatigue life of a bone; finally, the cracks reach a critical size, coalesce leading to fracture. (416)

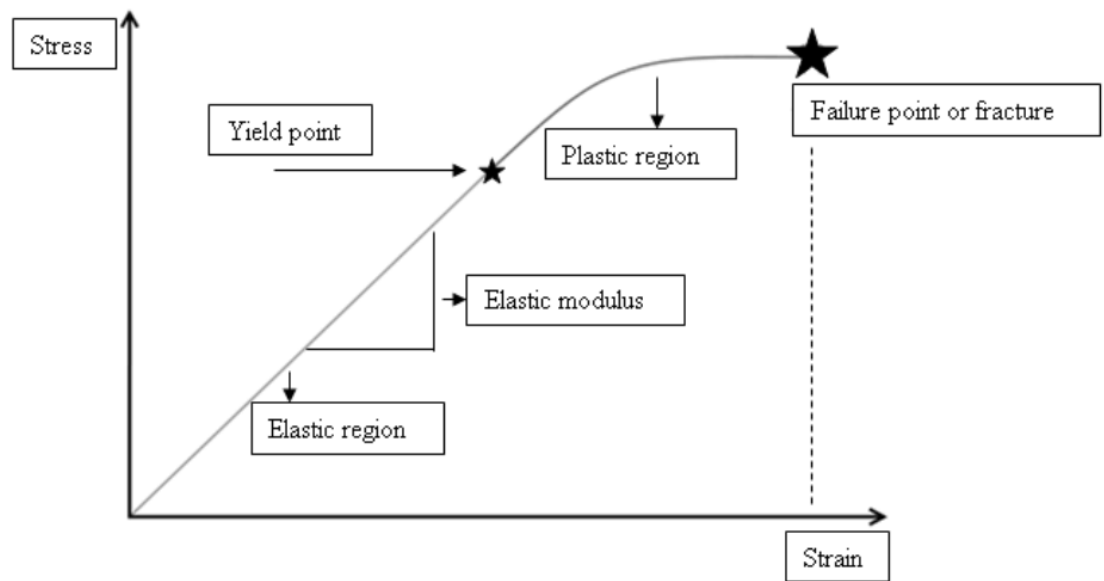


Figure B Stress Strain Curve

Appendix C Studies of cortical bone structure and strength in men

Table C Summary of studies on cortical bone structure and strength in men

Authors and year of publication	Study design	Population	Bone	Measures of bone geometry	Predictors
Russo et al. 2003 (98)	Cross-sectional	1205; 512 Italian men; aged 20 to 102	Tibia	TA, MA, CA, minimum moment of inertia	Age
Riggs et al. 2004 (97)	Cross-sectional	696; 323 White men; aged 20 to 97	Radius, tibia, femur	TA, MA, CA	Age
Lauretani et al. 2008 (106)	Longitudinal	809; 345 Italian men; aged 21 to 102	Tibia	TA, MA, CA, CSMI	Age
Yuen et al. 2010 (100)	Cross-sectional	1258; 620 Chinese men; aged 20 to 98	Radius and tibia	MA, CA, CSMI, and section modulus	Age
Sigurdsson et al. 2006 (99)	Cross-sectional	1715; 807 Icelandic men; aged 67 to 93	Femur	CA, CrtThk, bending strength, compressive strength	Age
Kaji et al. 2005 (103)	Cross-sectional	482; Japanese 230 men; aged 26 to 84	Radius	CA, CrtThk, periosteal circumference, endosteal circumference, SSI	Age, grip strength, smoking
Ward et al. 2010 (104)	Cross-sectional	728 White men; aged 40 to 79	Radius	TA, MA, CA	Age, hormones

TA = total bone area; some studies have assessed periosteal circumference instead

CA = cortical area; some studies have assessed cortical thickness instead

MA = medullary area; some studies have assessed endosteal circumference instead

CSMI = cross-sectional moment of inertia

SSI = strength strain index

Appendix D Studies examining inflammation markers and fractures and/or BMD

Table D Summary of studies examining the relationship between inflammation markers and fractures and/or BMD and bone strength

Authors	Design	Population	FU	Outcome	Predictor	Covariates	Methods	Conclusion
Sara Ahmadi-Abhari et al. 2013 (277)	Longitudinal	EPIC-Norfolk 18000 40 – 79 yr old men and women;8323 men	4.8	Incident fractures	hsCRP	age, sex, BMI, lifestyle, medical history, h/o fractures	Cox Proportional HRs	U-shaped association between fracture risk and hsCRP
Barbour et al. 2012 (268)	Nested case control	400 cases and controls from WHI aged 50 to 79		Hip fractures	IL6, TNF	BMI, physical activity, parental h/o fracture, smoking, alcohol use, etc.	Conditional logistic regression	Having elevated levels of 3 receptors in Q4 increased hip fracture risk 2 times or 1SD decrease in BMD.
Berglundh et al. 2015(417)	Longitudinal	OPRA, 1099 elderly women aged 75 and over	11.6	Fractures	CRP	weight, BMD	Cox Proportional HRs, ANCOVA, multiple regression, Kaplan-Meier	Low risk of major fracture in highest Q of CRP even after adjustment; persistent elevation of CRP associated with increased bone loss but not fracture
Cauley et al. 2007(267)	Longitudinal	Health ABC 70 – 79 yr old men and women, 2985	5.8	Incident fractures	hsCRP, TNF, IL, TNF and IL6 soluble receptors	age, sex, race, hip BMD, weight, smoking, fall history, weight gain, weight loss, physical activity, medications,	Cox Proportional HR	In healthy older adults, high levels of inflammation markers predicted fractures independent of frailty, weight loss and medications; IL6 likely operates via physical function
Ding et al. 2008 (273)	Longitudinal	Tasmania Older Adult Cohort Study, 168 older individuals 50 - 79	2.9	annual percent BMD change	change in and baseline hsCRP, IL6, TNF, urinary PYR/Cr ratio	age, sex, weight, height, smoking, other diseases	Multiple linear regression, logistic regression Q4 vs Q1to3	IL6 showed the most consistent association
Eriksson et al. 2014(274)	Longitudinal	MrOS Sweden, 2910 men aged 69 to 81	5.4 yrs	Fracture	hsCRP	aBMD, height, weight, calcium intake, physical activity, grip strength, smoking,	Cox Proportional HRs, ANCOVA	Low grade inflammation associated with increased fracture risk

Table D continued

						alcohol, medical history, medication use		
Ishii et al. 2013(266)	Longitudinal	SWAN, 1872 42 to 53 yrs	10 yr	Bone strength, Incident fracture	hsCRP	BMI	Multiple linear regression, Cox Proportional HRs	hsCRP and composite strength indices inversely related; presence of a threshold for CRP
Kim et al. 2007(418)	Cross-sectional	189 Korean pre and post-menopausal women	NA	NTx, BALP	hsCRP	age, BMI, years since menopause	ANCOVA	Ntx and BALP elevated in pre and post-menopausal women with hsCRP > 1.6mg/l
Koh et al. 2005 (307),	Cross-sectional	3662 pre and 1031 post-menopausal Korean women	NA	BMD	hsCRP	ALP, age, BMI, duration of menopause	ANCOVA	HsCRP positively correlated with ALP,
Nakamura et al. 2011(265)	Longitudinal	751 Japanese women aged 69 and over, Muramatsu Study	6	Incident fractures	hsCRP	age, BMI, forearm BMD, calcium intake, 25OHD, postural sway, osteoporosis medication, physical activity	Cox Proportional HRs	HsCRP associated with incident fractures
de Pablo et al. 2012 (276)	Cross-sectional	NHANES, 5261 men, 10475 women aged 20 and over		BMD	hsCRP	age, ethnicity, poverty, smoking, alcohol, physical activity, BMI, GFR, hormone use, DM, HT, COPD, CVD, CHF, stroke, medications, Vit D	Multiple linear regression	BMD and hsCRP dose-dependent relationship, the differences across quintiles in BMD is 2-3% similar to Vit D and calcium supplementation
Pasco et al. 2006(263)	Longitudinal	Geelong Osteoporosis Study, 522 women aged 65 and over	5.5 yrs	Fracture	hsCRP	C-telopeptide, Bone-ALP, BMD, prevalent fracture, age, anthropometry, lifestyle, medication use, comorbidity	Cox Proportional HRs	Dose response between hsCRP and fractures
Rolland et al. 2012 (275)	Cross-sectional	1149 men 19 – 87 yrs, Strambo Study	NA	Microarchitecture	hsCRP	Age, weight, height, 17 beta-estradiol, calcium supplementation, PTH, interaction between PTH and calcium supp	ANCOVA, Loess smoother,	TbSp was higher in men over 72 yrs in relation to hsCRP. Microarchitecture impairment does not explain hsCRP and fracture risk
Schett et al. 2006(264)	Longitudinal	Bruneck, 1000 (500 each) men and women aged 40 to 70		lifetime fractures	hsCRP	Age, sex, FU, income, smoking, alcohol, physical activity score, DM, BMI, creatinine, bone metabolism markers	Pooled Logistic regression, rate ratios	hsCRP independent predictor of nontraumatic fractures. Dose-response seen.

Table D continued

Sponholtz et al. 2014(308)	Cross-sectional	2915 men and women; 1293 men; Framingham Offspring Study, average age 61, middle-aged to older men and women	BMD	hsCRP, IL6, TNF	age, sex, height, weight, physical activity, smoking	Multiple linear regression	Inverse relationship with hip BMD only in premenopausal women. No relationship in men. Inflammatory markers not important.
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